EVALUATION OF CHROMAGAR AND PCR FOR DETECTION OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) FROM CLINICAL ISOLATES

Dissertation submitted in partial fulfillment of the Requirement for the award of the Degree of

M.D. MICROBIOLOGY

(BRANCH IV)

DEPARTMENT OF MICROBIOLOGY

TIRUNELVELI MEDICAL COLLEGE,

TIRUNELVELI - 627011.

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CHENNAI.

APRIL 2013.

CERTIFICATE

This is to certify that the dissertation entitled, **''Evaluation of Chromagar and PCR for detection of Methicillin Resistant *Staphylococcus aureus (MRSA)* from clinical isolates"** by **Dr.T.Susitha**, Post graduate in Microbiology (2010-2013), is a bonafide research work carried out under our direct supervision and guidance and is submitted to The Tamilnadu Dr. M.G.R. Medical University, Chennai, for M.D. Degree Examination in Microbiology, Branch IV, to be held in **April 2013**.

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CERTIFICATE

This is to certify that the Dissertation titled **''Evaluation of Chromagar and PCR for detection of Methicillin Resistant *Staphylococcus aureus (MRSA)* from clinical isolates"** presented herein by **Dr.T.Susitha** , is an original work done in the Department of Microbiology, Tirunelveli Medical College Hospital, Tirunelveli for the award of Degree of M.D. (Branch IV) Microbiology under my guidance and supervision during the academic period of 2010 - 2013.

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DECLARATION

I solemnly declare that the dissertation titled **"Evaluation of Chromagar and PCR for detection of Methicillin Resistant *Staphylococcus aureus* (MRSA) from clinical isolates"** is done by me at Tirunelveli Medical College hospital, Tirunelveli.

The dissertation is submitted to The Tamilnadu Dr. M.G.R.Medical University towards the partial fulfilment of requirements for the award of M.D. Degree (Branch IV) in Microbiology.

Place: Tirunelveli
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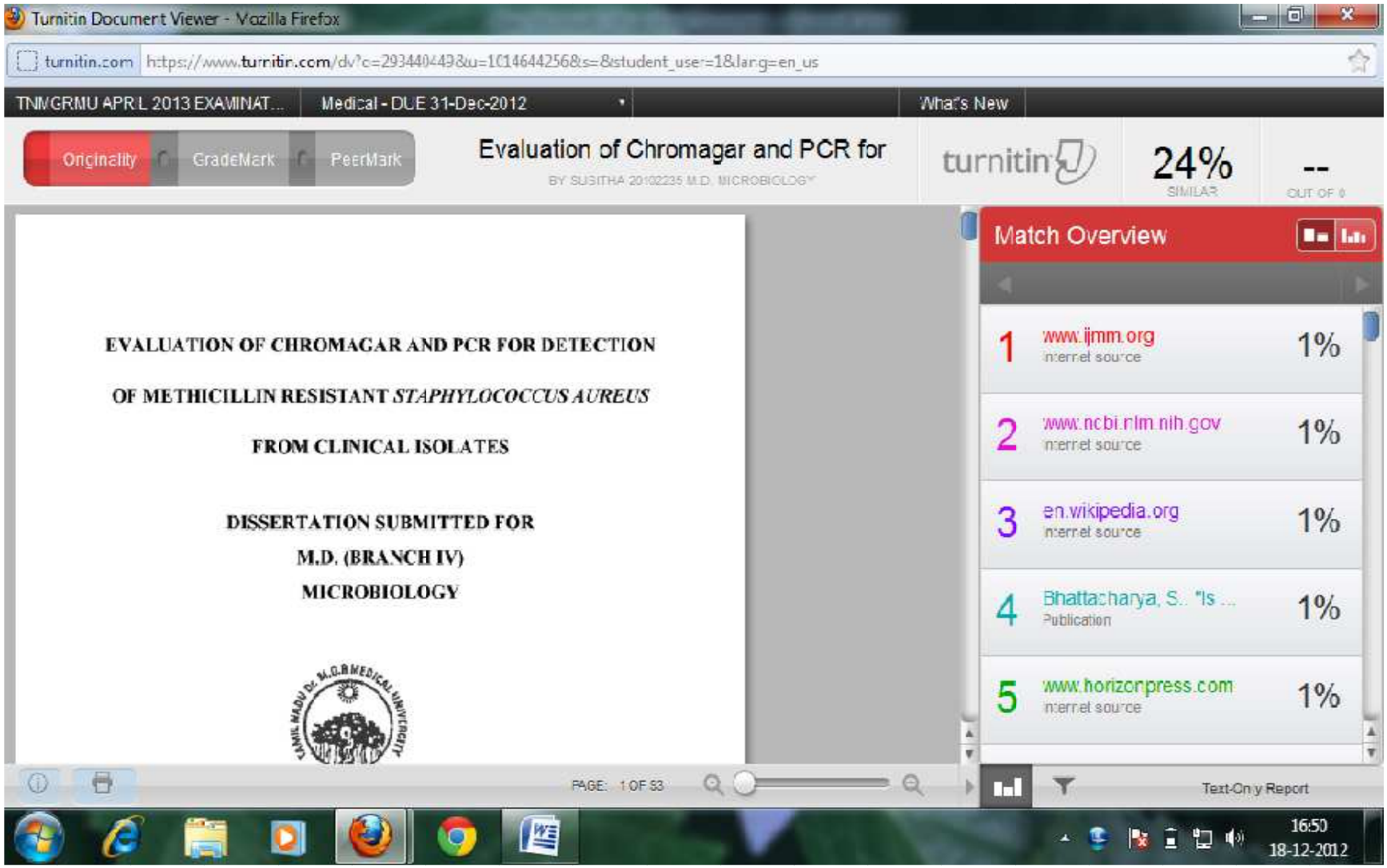
Contents

CONTENTS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This is to certify that the Institutional Ethical Committee of this College unanimously approves the Thesis /Dissertation/ Research Proposal submitted before this committee by Dr.T.SUSITHA, **A POST GRADUATE IN MICROBIOLOGY,** Department of MICROBIOLOGY of Tirunelveli Medical College /Hospital, Tirunelveli titled **"EVALUATION OF CHROMAGAR AND PCR FOR DETECTION OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) FROM CLINICAL ISOLATES"** registered by the IEC as 201/MICRO./IEC/2012 dated. 11.07.2012. The Investigator is hereby advised to adhere to all the stipulated norms and conditions of this ethical committee.

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Introduction

1. INTRODUCTION

The emergence of antibiotic resistance is a health problem worldwide and has affected the management and outcome of wide spectrum of infections. It contributes to significant mortality and morbidity and remains a hinderance to the control of infectious diseases. It leads to increase in health associated expenses and also acts as a barrier in the healthcare security of countries.[1] Now-a-days, the need for newer antibiotics to treat infections caused by Gram positive organisms is being increasingly felt.

Globally, *Staphylococcus aureus (S.aureus)* is considered as one of the most common cause of nosocomial infections. This remains as the hardiest of the non-sporing bacteria and can survive well in the environment under both moist and dry conditions. The high prevalence of *S.aureus*, together with its propensity to infiltrate tissues, colonize foreign body material, form abscesses and produce toxins, makes it by far the most feared micro-organism in healthcare-associated infections.

In recent times, there is a steady rise in the number of *S.aureus* isolates that show resistance to Methicillin and has evolved as a serious problem since resistance to this drug indicates resistance to all β-lactam antibiotics. Multiple use of antibiotics and prolonged hospitalisation are important factors which make hospital an ideal place for transmission and perpetuation of Methicillin Resistant *S.aureus* (MRSA).[2] For these above

reasons, accuracy and promptness in the detection of Methicillin resistance plays a key role for good prognosis of infections and hence abrupting its transmission.[3]

1.1. Historical importance

Sir Alexander Ogston, a Scottish surgeon in 1880, showed that a number of human pyogenic diseases were associated with a cluster-forming micro-organism and introduced the name *'Staphylococcus'*. In Greek, *'staphyle'* means bunch of grapes and *'kokkos'* means berry. Von Daranyi, in 1925 was the person to identify the coagulase test for *S.aureus*.[4]

1.2. Morphology

Staphylococci are placed in the family *Bacillaceae* of the order *Bacillales*. *S.aureus* is a Gram positive, uniformly spherical cocci of 0.5µm to 1.5µm in diameter on light microscopy and tends to occur in irregular grape-like clusters and less often, singly, pairs, tetrads, and short chains. This is due to the incomplete cell division in three perpendicular planes. In liquid media, singles, pairs and short chains are also seen. They are facultative anaerobes, nonmotile, non-sporing, and catalase positive.[5]

1.3. Cultural characteristics

Colonies of *S.aureus* are medium to large, smooth, low convex, entire, glistening, densely opaque and of butyrous consistency and are β- hemolytic on sheep blood agar at 37˚C when incubated for 18- 24

hours. The colonies of *S.aureus* are usually deep golden yellow (*aureus* means golden) and pigmentation can be enhanced on fatty media such as Tween agar, by prolonged incubation and at room temperature. On Mannitol salt agar it forms 1mm diameter yellow colonies surrounded by yellow medium due to acid formation.[5]

1.4. Biochemical reactions

S.aureus ferments a range of sugars of which the significant one is mannitol. Acetoin production, gelatinase and alkaline phosphatase are all typically positive. Indole is negative while urease and lactose fermentation are variable characters. It produces a deoxyribonuclease and a thermonuclease.[5] *S.aureus* gives a positive test for bound coagulase (clumping factor). It produces free coagulase which clots plasma by converting fibrinogen to fibrin and this property is used as a criterion in clinical laboratories to diagnose pathogenic *S.aureus*.

1.5. Habitat

S.aureus is found in the anterior nares of 20-40% of the adults and also in the intertriginous skin folds, the perineum, the axilla and the vagina.[6]Decreased ciliary action and attachment to cell associated and cell free secretions favour its adhesion to nose.[7]

1.6. Pathogenesis

The bacterium can form biofilms, the tool which helps in the invasion of the defense mechanisms. The microcapsule of this bacterium has 'zwitterionic' characters and also paves way for formation of abscess.[8]The protein A of *S.aureus* attaches to the Fc portion of immunoglobulin and by this process opsonization can be inhibited. *S.aureus* produces leukocidins which leads to the production of pores in the cell membrane and hence lysis of the leukocyte.[9]

During infection, enormous enzymes are released, such as proteases, lipases and elastases which directs its progression to ultimate destruction. Some isolates produce superantigens, which produces "cytokine storm", resulting in food poisoning, scalded skin and toxic shock syndrome.[10]

1.7. Mode of transmission

Nasal carriers of *S.aureus* have a three to six time's higher risk of nosocomial infection than non-carriers.[11] *S.aureus* is transmitted from person to person by direct contact, fomites, air or unwashed hands of health care workers in nosocomial setting. Respiratory droplets and skin squames released from the patients are other possible mechanisms for MRSA transmission in hospitals.[12] When newborns are colonized by these organisms, the nursing mothers are at risk of developing mastitis.[13]

1.8. Infections

S.aureus may cause a variety of infections ranging from mild to life-threatening serious illnesses. Infections generally involve intense suppuration and necrosis of tissue. This organism is frequently isolated from postsurgical wound infections.[6] *S.aureus* can be recovered from almost any clinical specimen. The infections[14] caused by this organism are as follows:

- Skin and soft tissue- Impetigo, boils, carbuncles, abscesses, cellulitis, fasciitis, pyomyositis, surgical and traumatic wound infections.
- Foreign body associated- Intravascular catheter, urinary catheter, surgical implant, endotracheal tubes.
- Intravascular- Bacteraemia, sepsis, septic thrombophlebitis, infective endocarditis.
- Bone and joints- Septic osteomyelitis, septic arthritis.
- Respiratory -Pneumonia, empyema, sinusitis, otitis media.
- Other invasive infections- Meningitis, surgical space infection.
- Toxin mediated diseases- Staphylococcal toxic shock syndrome, food poisoning, staphylococcal scalded skin syndrome, bullous impetigo, necrotizing pneumonia, necrotising osteomyelitis.

1.9. Risk factors

S.aureus can act as a significant opportunistic pathogen under the following conditions[6] given below:

- Defects in leukocyte chemotaxis, either congenital or acquired like Job's Syndrome or diabetes mellitus.
- Defect in opsonization by antibodies.
- Defects in intracellular killing of bacteria following phagocytosis.
- Skin injuries like burns, surgical incisions, eczema etc.
- Presence of foreign bodies like sutures, intravenous line etc.
- Infection with other agents, particularly viruses.
- Chronic underlying diseases such as malignancy, alcoholism.
- Therapeutic or prophylactic antimicrobial administration.

1.10. Evolution of MRSA

Oxacillin and Methicillin are semisynthetic Penicillins that are stable to staphylococcal β-lactamase by virtue of the strategic placement of certain side chains on the molecule. These drugs were developed specifically for the treatment of infection caused by β -lactamase producing *S.aureus.* In 1959, the drug Methicillin was introduced and the bacterium just needed six months to create resistant strains to it.[15]

1.11. Mechanism of resistance

1.11.1. Penicillin Binding Proteins

Under normal conditions, five Penicillin Binding Proteins (PBP) namely PBP1, PBP2, PBP2B, PBP3 and PBP4 are produced by the Methicillin Susceptible *S.aureus* (MSSA) isolates.[16]But an additional one, PBP2a is produced by the Methicillin resistant isolates and they differ from other PBPs, in the low affinity exhibited towards the β-lactam antibiotics.

1.11.2. Staphylococcal Cassette Chromosome *mec*

Methicillin resistance is conferred by the *mecA* gene, which is a part of a mobile genetic element called Staphylococcal Cassette Chromosome (SCC) *mec*. SCC*mec* is flanked by cassette chromosome recombinase genes (*ccrA/ccrB* or *ccrC*), that allow transmission of SCC*mec*.[10] Currently, six unique SCC*mec* types (I-VI) ranging in size from 21–67 kb have been identified and are distinguished by the variation in *mec* and *ccr* gene complexes.[17]

1.11.3. The *mecA* gene

The *mecA* gene encodes the 78-kDa PBP2a.[18] *The mecA* is under the control of two regulatory genes, *mecI* and *mecR1*. *mecI* is usually bound to the *mecA* promoter and functions as a repressor. In the presence of a β-lactam antibiotic, mecR1 initiates a signal transduction cascade that leads to transcriptional activation of *mecA*.[19]

1.12. Hospital acquired-MRSA and Community Acquired-MRSA

Hospital acquired (HA)-MRSA is usually associated with persons who have had frequent or recent contact with hospitals or other long-term care facilities such as nursing homes and dialysis centers. Community acquired (CA)-MRSA was isolated from indigenous Australian patients.

Table - 1.1

Characters of HA-MRSA and CA-MRSA strains[15]

Character	HA-MRSA	CA-MRSA
Clinical presentation	Invasive and commonly surgical site infections	Rarely invasive and commonly skin and soft tissue infections
Predominant age	Old aged	Young people
Target group	Immuno-compromised	Healthy persons
Antibiotic resistance	Multi-drug resistant	β-lactam resistant
Resistance gene	SCC*mec* I-III	SCC*mec* IV, V
Presence of PVL	Absent	Present

1.13. Laboratory diagnosis

Disc diffusion (DD) methods are the most widely followed procedures, in routine clinical laboratories. The acronym MRSA, is still

followed due to its historic role. The drugs Oxacillin and Cefoxitin are tested instead of Methicillin because:

- ❖ Methicillin is not manufactured now-a-days.
- ❖ Oxacillin maintains its activity better during storage.
- ❖ More likely to detect heteroresistant strains.

1.13.1. Heteroresistance:

Although, both susceptible and resistant cells are present in the culture, only a small number of cells express the resistance. Conditions that favour the heteroresistance are :

- ❖ Neutral p^H
- ❖ Cooler temperatures (30–35˚C)
- ❖ Presence of NaCl (2–4%)
- ❖ Prolonged incubation (up to 48 hours).

The following methods are standard ones for detecting Methicillin resistance as per The Clinical and Laboratory Standards Institute (CLSI)[15]

- ❖ Cefoxitin disc test
- ❖ Latex agglutination test
- ❖ Oxacillin screen agar.

1.13.2. Oxacillin DD method

Good visual interpretation with Oxacillin disc, may help in the detection of highly heteroresistant strains. Most isolates are deemed as sensitive, due to the hazy zones produced. This method can't be relied due to its lower specificity.[18]

1.13.3. Oxacillin screen agar

Although this test is called a "screen" the results can be considered definitive for assessing Oxacillin resistance in *S. aureus.* The sensitivity of this method, approaches 100% for the detection of MRSA.[18]

1.13.4. Cefoxitin DD method

DD by Cefoxitin is easy to predict than other conventional methods. Only the isolates exhibiting *mecA*-mediated resistance are strongly induced and are reliably picked up by this method.[20] However, non-*mecA* mediated Methicillin resistance in *S. aureus* is a rare occurrence.

1.13.5. Broth dilution method

Though considered as a standard test for MRSA, this method has been replaced by the molecular techniques. More than 90% of the resistant strains are detected by the broth micro dilution method under appropriate conditions.[18]

1.13.6. E-test

The E-test method has the advantage of being easy to perform, as a disk diffusion test and its accuracy approaches that of PCR.[21]

1.13.7. Latex agglutination test

This method involves extraction of PBP2a from colonies and their detection by agglutination with latex particles coated with monoclonal antibodies to PBP2a. These tests are accurate and are faster than the conventional methods. Latex tests involves lysis/extraction, centrifugation to pellet cellular debris and mixing of the supernatant with the test and control latex reagents.[6]

1.13.8. Chromagar

In recent years, the chromogenic media has been emerging as a boon, for the reliable and faster detection of Methicillin resistant isolates. These media allow direct colony color-based identification of the bacteria and thus is an upcoming technique. This saves time in subculturing the isolate and further reactions and is indeed the need of the hour.

1.13.9. Automated systems

Automated systems have definitive role in the diagnosis of the Methicillin resistant isolates but sensitivity is not equal to that of the standard procedures.[18]They are:

- ❖ Microscan conventional panels (Dade Behring)
- ❖ Phoenix (Becton Dickinson)
- ❖ Vitek (bioMerieux)

1.13.10. Polymerase chain reaction

Polymerase chain reaction (PCR) is considered the “gold standard” for detection of Methicillin resistant isolates. The detection of non-expressed *mecA* along with its rapid techniques makes it a reference technique in the laboratories for detection of Methicillin resistance. Recently addition of a second gene in addition to *mecA*, helps in the detection of resistance to various antibiotics among MRSA isolates.

1.13.11. GeneXpert

The target of the assay, is the junction of the SCC*mec* cassette and *orfX*.[22]The test is easy to follow and could be performed within five minutes and is therefore suitable for MRSA point of care testing.[23]

1.13.12. Phage typing

Strains of *S.aureus* can be differentiated into different phage types by observation of their pattern of susceptibility to lysis by a standard set of *S.aureus* bacteriophages. Virulent phages cause lysis of staphylococci and thus produce a clearing in the lawn of growth. Many strains of MRSA are non-typable with standard and additional phages.[13]

1.14. Control of MRSA

1.14.1. Need for control of MRSA

The control of MRSA, is important for the reasons given below:

- ❖ High transmission.
- ❖ Treatment with multidrugs are expensive.
- ❖ Side effects are higher.
- ❖ Poorer prognosis.
- ❖ Limited number of oral agents available.[24]

1.14.2. Control measures

Hand hygiene

Alcohol-based hand rubs/gels or using soap and water should be adhered strictly. This is the initial and major step in preventing transmission.

Patient isolation

An infected or colonized patient should be placed in separate rooms as far as possible and barrier precautions are to be followed.

Contact precautions

The health-care provider should wear gloves, apron and adhere to strict hand hygienic procedures.

Droplet precautions

Surgical masks are to be worn when the need to work closely with the patient arises. In patients with skin exfoliative lesions, masks are advised during bed making.

Decolonization of patients/ carriers

Eradication of MRSA carriage is not always successful. Topical intranasal mupirocin and fusidic acid are to be installed.

Environmental cleaning

Regularly clean with an all-purpose detergent and water and make sure that all horizontal surfaces are damp dusted and floors vacuumed.

The incidence of Methicillin resistance is a growing problem in the hospitals worldwide. Accurate and speedy techniques are vital for treating, managing, and preventing MRSA infections. Effective detection of MRSA can be difficult in simple clinical laboratories because susceptible and resistant populations may coexist in the same culture. Conventional methods are numerous and the choices in selection and application varies, among laboratories. Many phenotypic methods fail to detect Methicillin resistance and the sensitivity pattern of the isolates remains unpredictable among hospitalized patients. So a faster and cost-effective ideal method, which detects all MRSA strains is of utmost necessity. With this background, this study is undertaken to assess the prevalence, antimicrobial sensitivity patterns and to evaluate various

conventional and molecular methods for effective MRSA detection among clinical isolates.

Aim and Objectives

2. AIMS AND OBJECTIVES

2.1. To study the antimicrobial sensitivity pattern of *S.aureus* among pus samples at Tirunelveli Medical College, Tirunelveli.

2.2. To determine the prevalence of MRSA among the clinical isolates.

2.3. To evaluate Chromagar for detection of MRSA.

2.4. To confirm the MRSA isolates by Real- Time PCR for *mecA* gene.

Review of literature

3. REVIEW OF LITERATURE

"Antibiotic resistance in *S.aureus* was not known when Penicillin was first introduced in 1943, by Alexander Fleming who observed the antibacterial activity of the penicillium mould against a culture of *S.aureus.*[25] *S.aureus* remains as one of the most dangerous nosocomial pathogens. MRSA is the strain of *S.aureus* that had developed, through the process of evolution, resistance to β-lactam antibiotics.

The resistance of MRSA to more common antibiotics makes it a difficult organism to be handled and thus are more dangerous. The association of multidrug resistance with MRSA adds to the problem and it is rightly said that "hospital dust is most dangerous than roadside dust" and the danger is from MRSA.[26]

3.1. Epidemiology

The resistance of *S.aureus* to Methicillin varies from region to region and is also not similar at different times in the same hospital. MRSA has been reported all over the world. MRSA has emerged globally in the last three decades, especially within hospital settings.

3.1.1. Global scenario of MRSA

In 1961, Jevons did screening of 5000 clinical isolates and identified three MRSA isolates from England.[27] In United States, the first outbreak of MRSA occurred in 1968, at the Boston City Hospital.

Blot et al 2002, had found more deaths among MRSA bacteremia than MSSA.[28] In United States, 50% of hospital acquired infections in ICUs are due to MRSA.[29]

According to a European Antimicrobial Resistance Surveillance System report, MRSA was held responsible for 0.5 to 44% of cases of staphylococcal bacteremia in Europe and the highest incidence of 44% in Greece and lowest of 0.5% in Iceland.[30]

In 2010, encouraging results from a CDC, showed that life-threatening MRSA infections are declining. Invasive MRSA infections that began in hospitals decreased 28% from 2005 to 2008. Decreases in infection rates were even more for patients with bloodstream infections. In addition, the study showed a 17% decrease in invasive MRSA infections of community onset in people with recent exposures to healthcare settings. This report complements data from the National Healthcare Safety Network. They found declining rates of upto 50% in bloodstream infections occurring in hospitalized patients from 1997 to 2007.[31]

3.1.2. MRSA in India

In Asia, MRSA averages 70% of hospital-acquired *S. aureus* isolates, but paucity of information remains from most regions. In India, the prevalence of MRSA is increasing drastically among hospitals, and is approximately 30% of *S. aureus* infections.[32]The reported incidence of

MRSA in India was found to range from 26% to 51.6%.[33] Overall the rate of Methicillin resistance among large hospitals in India with *S. aureus* is nearly 32%.[2]

A study by Verma et al[34] 2000, had shown the highest prevalence of 80.78% among 484 *S.aureus* isolates tested at Indore. Tahnkiwale et al[35] 2002, did a study from Nagpur on 230 *S.aureus* and found the prevalence of MRSA to be 19.56%. The study done by Mulla et al[36] 2007 at Surat, had shown the prevalence of MRSA among 135 staphylococci as 39.5%.

The prevalence rate was 7.5 to 41% among three hospitals in New Delhi. (Gadepalli et al[37] 2009).The study by Pal et al[38] 2010, from Jaipur stated that the prevalence of MRSA was 7% only, among *S.aureus* isolates. The study from Ujjain, found the prevalence to be 16% (Pathak et al[39] 2010).

3.1.3. MRSA in Tamil Nadu

Reports on MRSA isolates are very scanty in Tamil Nadu. So MRSA, remains an underestimated problem and effective measures are not a important measure in the hospital. Rajaduraipandi et al[40] 2006, from Coimbatore, found that the 250 (31.1%) were MRSA positive among 906 *S.aureus* isolates.

A study from Chennai, had screened 298 suspected septicemic children and isolated 54 bacteremic children. *S.aureus* constituted 26 of them and the prevalence of MRSA among them was 10 (38.46%).

(Saravanan et al[25] 2009) .

The study by Thangavel et al[41] 2011, from Namakkal revealed that 10 (7.9%) were MRSA out of 126 clinical isolates while the remaining were MSSA and coagulase negative staphylococci.

3.2. MRSA distribution according to age and gender

A higher prevalence rate was seen among females (60.86%) than in the males (39.13%) in the study by Sharma et al[42] 2011. Mathanraj et al[43] 2009, found that the male gender was a significant factor in the study conducted with 17 (8.5%) of MRSA isolates. Males had a prevalence of 12.4% (15/118) while females had 2.4% (2/82) only.

3.3. Risk factors

Initially, infections due to MRSA were almost acquired in healthcare settings. The most common risk factors associated with MRSA were recent antibiotic intake, admission to emergency care units, surgery, and exposure to another patient colonized with MRSA.

3.3.1. Nasal carriage

In the study by Kumar et al[7] 2011, the carriage rate of *S.aureus* was 33 among (82.5%) doctors and seven among laboratory technicians (17.5%) while that of MRSA was 15 among doctors (83.3%) and three among lab technicians (16.6%).

3.3.2. Prolonged stay at hospital and antibiotic therapy

The study by Srinivasan et al[44] 2006 found the following factors to be associated with MRSA: prolonged postoperative treatment, recent antibiotic use and emergency admissions in the hospital. Seventy percent of the isolates were from postoperative cases undergoing emergency surgeries. Isolation was more during the second week of hospital stay. Emergency admissions had a significant risk of chance of early isolation. Prior treatment with multiple antimicrobials (38%) was found to be another significant factor.

3.3.3. Old age and Diabetes

Huijer et al[45] 2008, found that most of the MRSA isolates from surgical units were from aged and diabetic patients. This reflects the waning effect of the immune system. This may be due to the delay in discharge and prolonged antimicrobial treatment at hospital which results in enhanced antibiotic pressure.

3.3.4. Race

A study by Sedik et al[46] 2009, from USA had found out that by race, African-American patients were most likely to acquire MRSA infections (47%), followed by Caucasians (35%), Hispanics (31%), and Asian/Pacific Islanders (24%).

3.3.5. HIV

HIV infected persons (14%) are at higher chance of acquiring MRSA infection than non-HIV infected (3%) ones. Prolonged intake of Co-trimoxazole has been reported to be associated with *S.aureus* colonization. Recent antibiotic intake, CD4 T cell count < 200/mm^3, presence of indwelling catheter, presence of skin lesions and prolonged stay at hospital are the risk factors associated with HIV to be infected by MRSA.[47]

3.4.6. Burns

Marked immunosuppresion with indwelling catheters and endotracheal tubes, longer admissions at hospitals and the open wound itself are important factors which favour MRSA acquisition.

The study by Matsumura et al[48] 1996, found a prevalence of 15% among adults and children in burns patients. In the study by Roberts et al[49] 1998, 39.4% of MRSA infections occurred in burns unit.

3.4.7. Surgery

Srinivasan et al[44] 2006, from PIMS found that surgical units accounted for 40 (80%) of the MRSA isolates when compared to the 10 (20%) in medical units. Hujer et al[45] 2008, showed that majority of the MRSA isolates was from surgical units.

3.4.8. Job's syndrome

This autosomal disorder presents with cold abscess which are prone for infections with *S.aureus* especially MRSA.[50]

3.5. Distribution on the basis of infection

The study by Mehta[51] et al 1998, made observation of the isolation rate of MRSA and found it to be 33% from pus and wound swabs. Quershi et al[52] 2004, found a high isolation rate of 83% MRSA from pus. Rajaduraipandi et al[40] 2006, from Coimbatore found that out of the 1847 pus samples, 575 (31.1%) were *S.aureus* isolates and MRSA isolates were found to be 193 (33.6%). The study done by Mulla et al[36] 2007, had shown that out of the total 20 *S.aureus* ,11 were found to be MRSA among pus samples, followed by blood (five MRSA among 11 *S.aureus*) and one MRSA isolate each from other samples.

The study by Thangavel et al[41] 2011, from Namakkal revealed that out of the total 48 (38%) samples from wound, three (30%) were MRSA and the 12 (24%) were MSSA among males while two (20%) were MRSA and the eight (16%) were MSSA among females .The study

revealed that out of the total 47 (37%) samples from pus, 15 (30%) were MSSA and three (30%) were MRSA among males while seven (14%) were MSSA and one (10%) was MRSA among females.

Terry Alli et al[53] 2012, from Nigeria revealed that out of 48 MRSA isolates, 12 (21.4%) were from the wound swab and eight (40%) from eye and ear swabs. Karami et al[21] 2011, studied 106 MRSA isolates, 51 (48%) strains isolated from tracheal aspirate, 26 (24.5%) strains from wound, 10 (9.4%) strains from blood cultures, and 19 isolates (17.9%) from other specimens.

3.6. Antibiotic resistance of MRSA isolates

A few and important hallmarks of drug resistance are discussed below.

3.6.1. Penicillin

At the end of 1940, hospitals in England and the USA reported that up to 50 % of *S. aureus* strains were resistant to Penicillin. In 1950, 40% of hospital *S. aureus* isolates were Penicillin resistant; and by 1960, this had risen to 80%.[21]

3.6.2. Co-trimoxazole

The use of this drug has a magnificient role as an alternative to Vancomycin in serious MRSA infections. Rajaduraipandi et al[40] 2006, found that 63.2% were resistant among MRSA isolates. The study Hujier et al[45] 2008, showed 32 (21.3%) showed resistance and 118 (78.7%)

isolates were sensitive. A total of 96% resistance were observed among MRSA isolates (n=27) to Vancomycin by Sarma et al[54] 2010.

3.6.3. Vancomycin

Vancomycin was discovered in the 1950s and was initially used to treat Penicillin resistant staphylococci and other Gram-positive bacterial infections. The first isolate of Vancomycin intermediate *S.aureus* (VISA) emerged in 1996, from Japan. Complete resistance to the drug was observed from a patient in 2002, from Michigan.[55]

3.6.4. Multidrug resistance

MRSA are considered resistant to all penicillinase-stable Penicillins and β-lactam agents. MRSA usually are resistant to multiple classes of agents including Macrolides, Lincosamides and Tetracyclines. They also can be resistant to Fluoroquinolones and Aminoglycosides.

In the mid of sixties, occurrence of multidrug-resistant MRSA was reported world wide including India. The ability of IS*431* elements, through homologous recombination, to trap and cluster resistance determinants with similar insertion sequence elements explains the multiple drug resistance that is characteristic of MRSA.[18]

The drugs Ciprofloxacin, Clindamycin, Gentamicin and Vancomycin should be initiated only after antibiotic sensitivity testing. It is not entirely certain why some strains are highly transmissible and persistent in healthcare facilities.

In the study by Tahnkiwale et al[35], multidrug resistance was evaluated and the following resistance was observed among MRSA isolates: 97% for Cotrimoxazole and 93.3% for Chloramphenicol. Only 6.66% of the isolates showed resistance towards Gentamicin. All isolates were found to be susceptible to Vancomycin.

Arora et al,[26] found that 73% of the MRSA strains were resistant to ≥ 3 drugs. Majority of the isolates were resistant to Cephalexin (80.9%) , followed by Gentamicin (72.2%), Ciprofloxacin (67.8%), Erythromycin (61.7%) and Amikacin (37.4%). A 100% sensitivity was observed to Vancomycin.

3.7. Evaluation of various methods in laboratory identification of MRSA

Diagnostic Microbiology laboratories play a pivotal role in identifying earlier, isolates of MRSA. The bacterium must be generally cultured initially, for performing the confirmatory or reference methods.

3.7.1. Role of temperature and duration in MRSA detection

Laboratory methods have been developed to enhance the expression of resistance in staphylococci. So supplementation of media with Nacl and extending the incubation time increases the detection rate.

A study from Delhi, compared Cefoxitin DD with Oxacillin DD method among 155 *S.aureus* isolates. Cefoxitin disc identified 54.54% MRSA isolates and Oxacillin disc method identified 48.39% only. There

was no difference in zone diameter at 18 hours and 24 hours of incubation. (Gupta et al[56] 2009).

Kluytmans et al[57] 2002, evaluated Chromagar for Methicillin resistance. The sensitivity at 24 hours was 58.6% and at 48 hours it was higher (77.5%). The specificity at 24 hours was 99.1% and at 48 hours it was lower (94.7%).

Hal et al[58] 2007 from Sydney, compared Chromagar with PCR. The sensitivity of Chromagar for MRSA detection increased 8% only, with extended incubation to 48 hours. Specificitiy was 99% at 24 hours. However, the specificity decreased with 48 hours of incubation.

3.7.2. Evaluation of Cefoxitin DD method

A study from Sweden, evaluated the performance of a Cefoxitin 30µg disc on Iso-Sensitest agar, for detection of MRSA. A total of 457 *S.aureus,* including 190 MRSA isolates were confirmed by PCR. They concluded that the Cefoxitin method was excellent, with a sensitivity of 100% and a specificity of 99%. (Skov et al[59] 2003)

In the study by Hujer et al[45] 2008, MRSA detected by the DD test and PCR assay were identical. Consequently, the sensitivity and specificity of Methicillin DD test as compared to *mecA* gene PCR are therefore 100% respectively. Similarly, the sensitivity and specificity of Cefoxitin DD method in detecting MRSA as compared to *mecA* gene PCR were 97% and 97.4% respectively.

The study by Bhat et al[60] 2008, collected 210 *S.aureus* isolates and tested them for MRSA by agar screen method and DD method. A total of 69 (33%) isolates were MRSA by agar screen method and 59 (28%) by DD method. The use of higher bacterial density and the presence of Nacl in the medium may help in the better detection of MRSA by agar screen method. They concluded that the disc method is unreliable for Methicillin resistance detection.

Rao et al[61] 2011 from Karnataka, revealed that out of the 300 *S.aureus* isolates, 50 were found to be MRSA by both Cefoxitin DD and PCR while 48 isolates only were picked up the Oxacillin DD method. The sensitivity and specificity of Oxacillin disc method was 90% and 100% respectively and the same for Cefoxitin disc method was 100% respectively and were in concurrence with the PCR for *mecA* gene. They concluded that Cefoxitin DD test can be used as an alternative to PCR.

3.7.3. Evaluation of Chromagar

A study from Switzerland, had compared four chromogenic media for their efficacy with PCR. Out of the 247 clinical isolates, 70 were found to be MRSA. The Chromagar identified a maximum of 64 of the MRSA isolates and a minimum of 37.The maximum and minimum sensitivity and specificity were 91% and 53% and 95% and 68% respectively.(Cherkaoui et al[62] 2007).

A study from UK, had compared Chromagar with PCR for effective MRSA detection. A total of 148 isolates (12.3%) were MRSA positive, of which 146 (12.1%) were PCR positive and 128 (10.6%) were Chromagar positive. A total of 126 (10.5%) were both PCR and Chromagar positive and 20 (1.66%) were positive by PCR only while two (0.2%) were positive by Chromagar only. They concluded that PCR is very much sensitive than Chromagar for MRSA detection.(Danial et al[63] 2011).

Karami et al[21] 2011, from Tehran did a study comparing Chromagar with E-test as gold standard. Out of the total 294 *S.aureus*, 106 (36%) were found to be MRSA. Chromagar showed 110 isolates as MRSA. The sensitivity and specificity for the Chromagar were 100% and 97.9% respectively and Positive Predictive Value (PPV) and Negative Predictive Value (NPV) were 96.3% and 100% respectively.

3.7.4. PCR

The study by Mehndiratta et al[64]2009, did typing of 125 MRSA isolates by bacteriophage and PCR-RFLP of *spa* gene. DNA sequencing analysis was performed and all the isolates had *mecA gene*. 52% were typeable and five patterns were observed. Among the non-typeable isolates, four different patterns were observed.

The study from Switzerland, analysed 1,601 specimens for MRSA detection by PCR. The sensitivity, specificity, PPV and NPV were 84.3%, 99.2%, 88.4% and 98.9% respectively.(Lucke et al[65] 2010)

The study from Saudi Arabia, had done multiplex PCR targeting 16sRNA, *PVL* and *mecA* gene among 101 isolates. All the isolates were positive for 16sRNA and *mecA* gene. Only 38, of the isolates (37.6%) gave positive results for *PVL* gene. The predominant type were SCCmec type V 43 (42.5%) and type III 39 (38.6%).(Moussa et al[66]2012)

3.8. Why are MRSA important?

- Causes serious life-threatening infections.
- Limited treatment options.
- MRSA are transmissible.

The high pathogenicity, the few number of treatment options available and transmission among hospitals are the major factors which make MRSA, to be considered as a threat to patients.

Materials and Methods

4. MATERIALS AND METHODS

The present study was conducted at the Department of Microbiology, Tirunelveli Medical College, Tirunelveli for a period of one year from September 2011 to August 2012 to assess the drug sensitivity pattern of *S.aureus* isolates from pus samples, to determine the prevalence of MRSA and to evaluate Methicillin resistance by Cefoxitin DD method, Chromagar and its confirmation by Real-Time PCR. Various risk factors associated with the study group, were statistically analysed and results were interpreted.

4.1. Materials

4.1.1. Sample collection and processing

A total of 100, non-duplicate *S.aureus* isolates from clinical pus samples were taken into the study. The *S.aureus* isolates were identified by:

- Morphology on Gram stained smear
- Colony appearance on nutrient agar
- Colony appearance on sheep blood agar
- Positive catalase test
- Positive tube coagulase test
- Sensitivity to Furazolidone (100µg)

4.1.2. Ethical clearance

As this study involved the clinical samples from the patients, ethical clearance was obtained before the commencement of the study.

4.1.3. Informed consent

Informed consent was obtained from all persons involved in the study.

4.1.4. Proforma

A filled in proforma was obtained from the patients with details like name, age, sex, ward, clinical diagnosis, risk factors, surgical intervention, hospital stay and other parameters relevant to the study.

4.1.5. Sample storage

The *S.aureus* isolates were sub-cultured on to nutrient agar slope and stored at 2 to 8˚C. The isolates were sub-cultured every month.

4.1.6 .Safety precautions

All the procedures were carried out in a Biosafety cabinet with due precautions.

METHODS

4.2. Antibiotic sensitivity testing

All the *S.aureus* isolates were tested by DD method to detect Methicillin resistance and their antibiotic sensitivity pattern.

4.2.1. DD method

DD method was performed by Kirby-Bauer method using Mueller Hinton agar with the following antibiotic discs (HiMedia Laboratories, Mumbai, India).

- Penicillin(10IU)
- Cefoxitin(30µg)
- Erythromycin(15µg)
- Clindamycin(2µg)
- Gentamicin(10µg)
- Amikacin(30µg)
- Ciprofloxacin(5µg)
- Cotrimoxazole(1.25/23.75µg)
- Vancomycin(30µg)
- Teicoplanin(30µg)
- Tigecycline(15µg)
- Linezolid(30µg)

Discs were stored in a tightly sealed container with dessicant at 2°C to 8°C. Before opening the container, discs were allowed to equilibrate to room temperature for one to two hours to minimize condensation and to reduce the possibility of moisture affecting the concentration of antimicrobial agents.

4.2.2. Mueller Hinton agar

The Mueller Hinton agar was purchased from HiMedia Laboratories, Mumbai, India and media was prepared according to the manufacturer's instructions (Appendix-I). Before inoculation, plates were dried by placing it in the incubator with their lids ajar, for 10–15 minutes.

4.2.3. Inoculum preparation

Inoculum was prepared by direct colony suspension method by taking four to five well isolated colonies of *S.aureus* from 18-24 hours culture, in Mueller Hinton broth to achieve a turbid suspension.

4.2.4. Inoculum standardization

The inoculum suspension was compared with 0.5 McFarlands standard suspension by positioning the tube side by side against a white card containing several horizontal black lines. The turbidities were compared by looking at the black lines through the suspensions. Once standardized, the inoculum suspension was used within 15 minutes of preparation.

4.2.5. Principle of DD test

The principle of DD depends on the formation of a gradient of antimicrobial concentrations as the antimicrobial agent diffuses radially into the agar. The drug concentration decreases at increasing distances from the disc. At a critical point, the drug concentration at a specific point

in the medium is unable to inhibit the growth of the test organism and the zone of inhibition is formed.

4.2.6. Procedure

- After standardization of bacterial suspension, the suspension was vortexed to make sure, it was well-mixed.
- Then by using a sterile swab, inoculation was done on Mueller Hinton agar and excess fluid was removed by pressing the swab against the side of the test-tube.
- Swab was streaked evenly over the surface of the medium in three directions; the plate was rotated approximately 60° for even distribution.
- With the petri dish lid in place, three to five minutes was allowed for the surface of the agar to dry.
- Using sterile needle mounted in a holder, the appropriate discs were evenly distributed on the inoculated plate.
- The discs were placed about 15mm from the edge of the plate and not closer than about 25mm from disc to disc.
- Only six discs were applied on a 90mm plate. Each disc was lightly pressed down to ensure its contact with the agar.
- The plate was inverted and incubated at 35˚C aerobically for full 24 hours.

4.2.7. Interpretation of results

After incubation, the inhibition zone was measured to the nearest millimeter using a ruler, under transmitted light. Inhibitory zone includes the diameter of the disc. After measuring, the millimeter reading for each antimicrobial agent was compared with that in the interpretive tables of the CLSI guidelines[67] and results were interpreted as either susceptible, intermediate or resistant. For Cefoxitin discs, zone size of $\geq$ 22mm was taken as sensitive while zone size of $\leq$ 21mm was taken as resistant. (Table 4.1).

Table.4.1. Interpretation of zone sizes

S. No	Antibiotic disc	Disc strength	Resistant (mm)	Intermediate (mm)	Sensitive (mm)
1.	Penicillin	10 IU	$\leq$ 28	-	$\geq$ 29
2.	Cefoxitin	30 µg	$\leq$ 21	-	$\geq$ 22
3.	Erythromycin	15 µg	$\leq$ 13	14-22	$\geq$ 23
4.	Clindamycin	2 µg	$\leq$ 14	15-20	$\geq$ 21
5.	Gentamicin	10µg	$\leq$ 12	13-14	$\geq$ 15
6.	Amikacin	30 µg	$\leq$ 14	15-16	$\geq$ 17
7.	Ciprofloxacin	5 µg	$\leq$ 15	16-20	$\geq$ 21
8.	Cotrimoxazole	1.25/23.75µg	$\leq$ 10	11-15	$\geq$ 16
9.	Vancomycin	30 µg	-	-	$\geq$ 15
10.	Teicoplanin	30 µg	$\leq$ 10	11-13	$\geq$ 14
11.	Tigecycline	15 µg	-	-	$\geq$ 20
12.	Linezolid	30 µg	-	-	$\geq$ 21

4.2.8. Quality control

The ATCC 25923 *S.aureus* strain, was included for each and every procedure performed.

4.2.9. D-test

This test was done to detect inducible Clindamycin resistance. It was done by placing both Erythromycin (15µg), and Clindamycin (2µg) discs on Mueller Hinton agar plate with a distance of 15 mm edge to edge. Following overnight incubation, flattening of the zone towards the Clindamycin disc with the shape of "D" indicated inducible Clindamycin resistance.

4.2.10. Other considerations

- ❖ All the isolates were confirmed for Vancomycin resistance by agar screen method.
- ❖ An isolate of MRSA is considered to be multidrug resistant if it shows resistance to ≥ 3 drugs, excluding Penicillin and Cefoxitin.

4.3. Chromagar

All the *S.aureus* isolates were inoculated onto Chromagar for detecting Methicillin resistance.

4.3.1. Principle

Chromogenic media detects the key microbial enzymes as diagnostic markers for pathogens through the use of "chromogenic"

substrates incorporated into a solid-agar-based matrix.[68] The chromogenic mixture incorporated in the medium is specifically cleaved by MRSA isolates to form bluish green coloured colonies.

4.3.2. Procedure

Four to five colonies of *S.aureus* from nutrient agar plate was streaked on the HiCrome MeReSa Agar with added MeReSa selective supplement (M1674 and FD299, *HiMedia Laboratories*, Mumbai, India) (Appendix-II) and incubated for 18-48 hours at 35ºC aerobically.

4.3.3. Interpretation

Appearance of luxuriant bluish green colonies on the HiCrome MeReSa agar indicated that the isolate was MRSA while the Methicillin sensitive *S.aureus* colonies, were inhibited. Observation for growth of the colonies were made at 24 hours of incubation. The plates showing negative results were further incubated for 24 hours and read for coloured colonies.

4.4. Real-Time PCR

The Methicillin resistant *S.aureus* isolates were further tested for *mecA* gene by Real-Time PCR by the kit purchased from Helini Biomolecules, Chennai, India and procedure followed according to the manufacturer's instructions.

4.4.1. Safety precautions

All the procedures were done in a Biosafety cabinet Level-2 with due precautions.

4.4.2. Equipments

- ❖ Vortex mixer
- ❖ Refrigerated centrifuge
- ❖ Thermo cycler (Biorad CFX 96)
- ❖ Computer for data storage

4.4.3. DNA extraction

Each silica based spin column recovered up to 20µg of DNA and yielded purified DNA of more than 30 kb in size. Isolated DNA was used directly for PCR reaction.

4.4.3.1. Components of extraction

- ❖ Lyophilised Proteinase K
- ❖ Proteinase K dilution buffer
- ❖ Lysis buffer
- ❖ Internal control template
- ❖ Wash buffer-I
- ❖ Wash buffer- II
- ❖ Isopropanol
- ❖ Elution buffer

4.4.3.2. Storage and stability

- ❖ The kit was stored at 25˚C.
- ❖ 1ml of Proteinase dilution buffer was added to each Proteinase stock vial. It was mixed well and stored at -20˚C.

4.4.3.3. Sample preparation

Four to five colonies of *S.aureus* grown on nutrient agar plate was inoculated into five ml of nutrient broth. It was incubated overnight at 35˚C. This was then transferred into three tubes, 1.5ml each. The tubes were then centrifuged for five minutes at 10,000 rpm. The supernatant was discarded and the bacterial pellet was stored at -20˚C.

4.4.3.4. Principle of extraction

Cells are lysed during a short incubation with Proteinase K in the presence of chaotropic salt, which immediately inactivates all nucleases. Cellular nucleic acids bind selectively to special glass fibres, pre-packed in the spin column. Bound nucleic acid is purified in a series of rapid “wash and spin” steps to remove contaminating cellular components. A special inhibitor buffer removes all salts and inhibitors from the preparations. Finally low salt elution releases the nucleic acids from the glass fibre.

4.4.3.5. Extraction procedure

- ❖ All the steps were done at room temperature.
- ❖ The bacterial pellet was suspended in 200µl of phosphate buffered saline and vortexed for 30 seconds.
- ❖ Lysis buffer of 400µl and 5µl of internal control template was added to the suspension.
- ❖ To the above suspension, 20µl of proteinase K was added.
- ❖ This was mixed immediately by inverting and incubated at 56°C for 15 minutes in a water bath.
- ❖ 200µl of Isopropanol was added and mixed well by inverting several times.
- ❖ Entire sample was pipetted into a spin column.
- ❖ This was centrifuged for one minute at 12,000 rpm. Flow through was discarded.
- ❖ 500µl of Wash buffer –I was added to the spin column.
- ❖ This was centrifuged for 60 seconds at 12,000 rpm. Flowthrough was discarded.
- ❖ 500µl of Wash buffer-II was added to the spin column.
- ❖ This was centrifuged for 60 seconds at 12,000 rpm and flow through was discarded.
- ❖ The steps with Wash buffer-II was repeated again.

- ❖ The flow through was discarded and centrifuged for an additional one minute at 12000 rpm to remove the residual ethanol.
- ❖ The spin column was transferred to a fresh 1.5ml microcentrifuge tube.
- ❖ 50µl of the Elution buffer (pre-warmed to 70˚C) was added to the centre of the spin column membrane. Care was taken not to touch the membrane with pipette tip.
- ❖ It was incubated for two minutes at room temperature and centrifuged for two minutes at 12,000 rpm.
- ❖ The column was discarded and purified DNA was stored at -20°C.

4.4.4. PCR amplification

4.4.4.1. Key ingredients for amplification

QPCR probe mix

The QPCR probe mix contains the essential components for PCR amplification like DNA polymerase and deoxynucleotides.

MRSA primer & probe mix

The MRSA primer & probe mix consists of TaqMan probe which is florescent labeled with FAM, forward primer and reverse primer.

Forwardprimer-ACTGCTATCCACCCTCAAACAG

Reverse Primer- CTGGAACTTGTTGAGCAGAGGTT

Internal Control primer & probe Mix

The internal control primer & probe mix consists of TaqMan probe which is florescent labeled with VIC, forward primer and reverse primer. The reason for including the internal control is to make sure that PCR inhibitors are not present in the extracted sample DNA and the performance of PCR mix ingredients are good. When no amplification was observed in internal control, it indicates that PCR inhibitors are present in the sample and efficiency of the nucleic acid purification is not optimum. It helps to rule out false negative results.

MRSA positive template

To be used for positive control mix.

Nuclease free water

For usage in negative control mix.

4.4.4.2. PCR amplification kit storage

The kit was stored at -20˚C.

4.4.4.3. MRSA reaction mix

The MRSA reaction mix for the samples consisted of QPCR 13µl, MRSA primer probe mix 2µl, internal control primer probe mix 1µl, purified DNA sample 5µl and a total volume of 21µl.(Table.4.2)

For positive control mix, 5µl of positive control template was added instead of sample DNA and for negative control mix, 5µl of nuclease free water was added instead of sample DNA.(Table.4.3& 4.4)

Initially negative control, followed by samples and finally positive control was added to prevent cross contamination. After adding all the ingredients, they were centrifuged and placed in the thermo cycler and the PCR reaction was allowed to occur.

Table.4.2.MRSA reaction mix for samples

S. No	Components	Volume
1.	QPCR probe mix	13 µl
2.	MRSA primer probe mix	2 µl
3.	Internal control primer probe mix	1 µl
4.	Purified DNA sample	5 µl
	Total volume	21 µl

Table.4.3.MRSA Positive control mix

S.No	Components	Volume
1.	QPCR probe mix	13µl
2.	MRSA primer probe mix	2µl
3.	Internal control primer probe mix	1µl
4.	Positive control template	5µl
	Total volume	21µl

Table.4.4.MRSA Negative control mix

S.No	Components	Volume
1.	QPCR probe mix	13µl
2.	MRSA primer probe mix	2µl
3.	Internal control primer probe Mix	1µl
4.	Nuclease free water	5µl
	Total volume	21µl

4.4.4.4. Basic steps in amplification

- **Initial denaturation** - First, the temperature is raised to 95˚C for four minutes for Taq enzyme activation.
- **Denaturation**- When the temperature is raised to 95˚C for 20 seconds, template DNA strand gets separated to two complementary strands.
- **Annealing**- When the temperature reduces to 55˚C for 20 seconds, two specific oligonucleotide primers binds to the DNA template complementarily.
- **Extension**- When the temperature rises to 72˚C for 20 seconds, DNA polymerase extends the primers at the 3' terminus of each primer and synthesizes the complementary strands along 5' to 3' terminus of each template DNA using deoxynucleotides in the reaction mixture. After extension, two single template DNA strands and two synthesized complementary DNA strands combine together forming two new double stranded DNA copies.

Each copy of DNA may serve as another template for further amplification. The products will be doubled each cycle. After 40 cycles, the final PCR products will have 2^n copies of template DNA. Data collection was done at the end of extension and the computer generates

the cross threshold (Ct) value by calculating the fluorescence emitted at the end of each cycle. (Table 4.5)

Table.4.5.Amplification profile for *mecA* gene

	Step	**Time**	**Temp**
	Taq enzyme activation	4 min	95^0 C
40cycles	Denaturation	20 sec	95^0 C
	Annealing/ Data collection	20 sec	55^0 C
	Extension	20 sec	72^0 C

4.4.5. Ct value

- ❖ When Ct value was less than 37, it was considered as positive for *mecA* gene.
- ❖ The test was repeated with Ct values between 37- 40.
- ❖ Negative result if no amplification occured. (Table 4.6)

Table.4.6. Interpretation of results

MRSA	**Negative control**	**Positive control**	**Interpretation**
Positive	Negative	Positive	Positive
Negative	Negative	Positive	Negative
Negative	Negative	Negative	Repeat
Positive	Positive	Positive	Repeat

Results

5. RESULTS

5.1. Study samples

The study was conducted at the Department of Microbiology, Tirunelveli Medical College, over a period of one year from September 2011 to August 2012. A total of 100 *S.aureus* isolates from pus samples were included in the study. These isolates were further tested for Methicillin resistance by Cefoxitin DD test, Chromagar and Real-Time PCR. The antibiotic sensitivity patterns of the isolates and the risk factors were further analysed.

5.2 Statistical Analysis

Data regarding the subjects were described in terms of percentages. The ages of the subjects were compared between the genders by student's unpaired't' test. The sensitivity, resistant and intermediately susceptible was described in terms of percentages. The multidrug resistance associated with Methicilin was interpreted by 'Z' test of proportions. The D-test was interpreted by paired chi-square test. The statistical procedures were performed with the help of the statistical software IBM SPSS statistics 20. The p values less than 0.05 was considered as significant ($p < 0.05$) in two tailed test.

5.3. Analysis by age and gender

Table 1. Sample distribution by age and gender

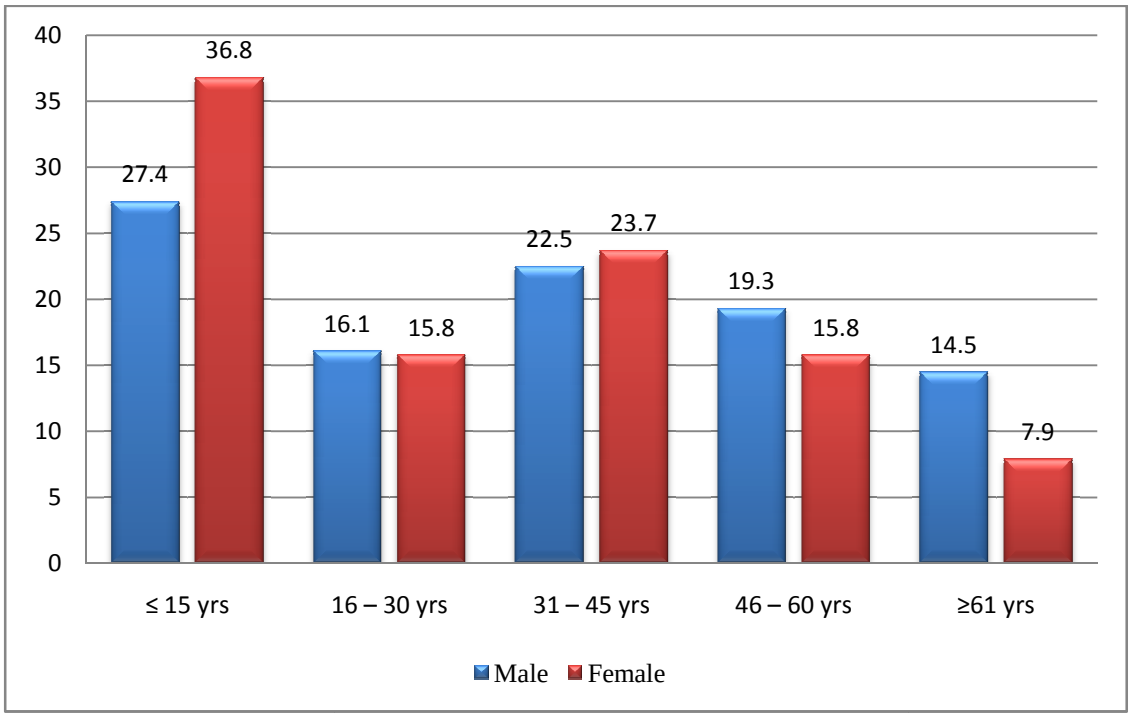
Age (years)	Male		Female		Total	
	No	%	No	%	No	%
≤ 15	17	27.4	14	36.8	31	31
16 – 30	10	16.1	06	15.8	16	16
31 – 45	14	22.5	09	23.7	23	23
46 – 60	12	19.3	06	15.8	18	18
≥61	09	14.5	03	7.9	12	12
Total	62	100	38	100	100	100

Out of 100 isolates, 62 isolates were from males and the remaining 38 isolates were from females. A total of 31 isolates, fell in the study group of ≤ 15 years of which, 17 isolates (27.4%) were from males and 14 isolates (36.8%) were from females. Out of the 16 isolates in the 16-30 years age group, 10 isolates (16.1%) were from males and six isolates (15.8%) were from females. A total of 23 the isolates were in the 31-45 age group, of which, 14 isolates (22.5%) were from males and nine isolates (23.7%) were from females. A total of 18 isolates were in the 46-60 years group, out of which 12 isolates (19.3%) were from males and six isolates (15.8%) were from females. Out of 12 isolates in persons above 61 years, nine isolates (14.5%) were from males and three isolates

(7.9%) were from females. The mean age of male was 36.9 years and that of female was 29.6 years and was not statistically significant.

(p> 0.05).(Figure.1)

Fig.1. Analysis of samples by age and gender



5.4. Analysis of various methods for Methicillin resistance

All the 100 *S.aureus* isolates, were evaluated for Methicillin resistance by Cefoxitin DD diffusion method and by growth on Chromagar. Of these, 34 isolates were resistant by Cefoxitin DD method. The Chromagar also showed the growth of the 34 isolates at 24 hours. No additional growth was observed at 48 hours of incubation.

These 34 Methicillin resistant isolates were confirmed for the presence of *mecA* gene by RT-PCR, which was considered as the “gold

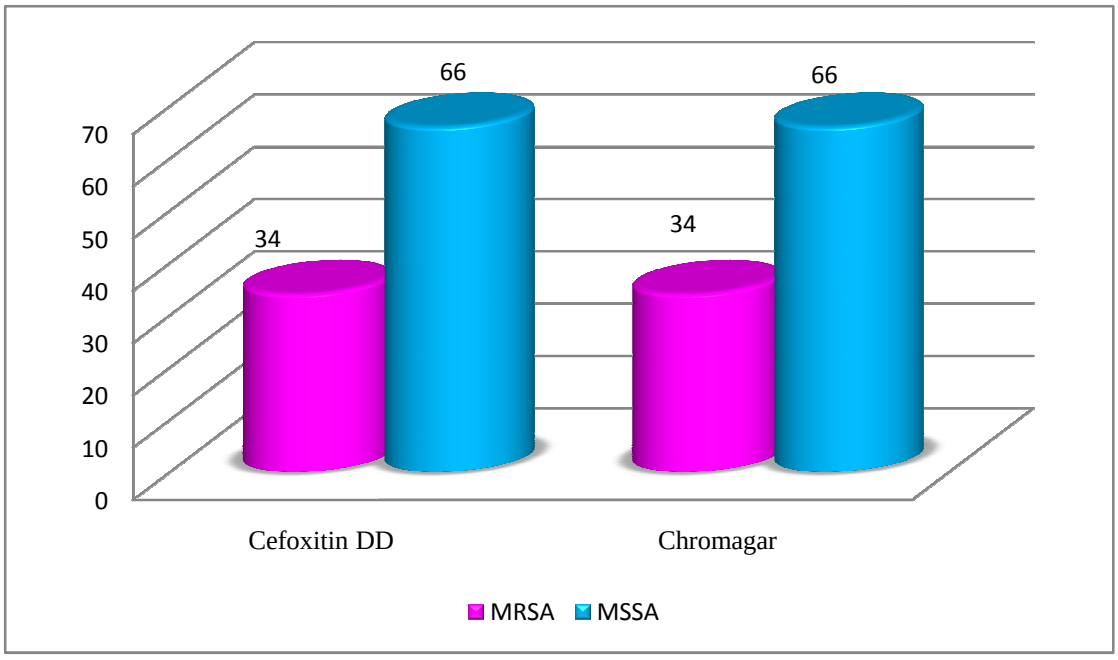
standard". The remaining 66 isolates were Methicillin sensitive by both Cefoxitin DD method and by Chromagar.(Table 2&3 and Figure 2&3).

5.4.1. Evaluation of cefoxitin DD method and Chromagar in detection of MRSA

Table 2.Comparison of Cefoxitin DD method and Chromagar

Method	MRSA		MSSA		Total
	No	**%**	**No**	**%**	
Cefoxitin DD	34	34	66	66	100
Chromagar	34	34	66	66	100

Fig.2 Methicillin resistance by Cefoxitin DD method and Chromagar



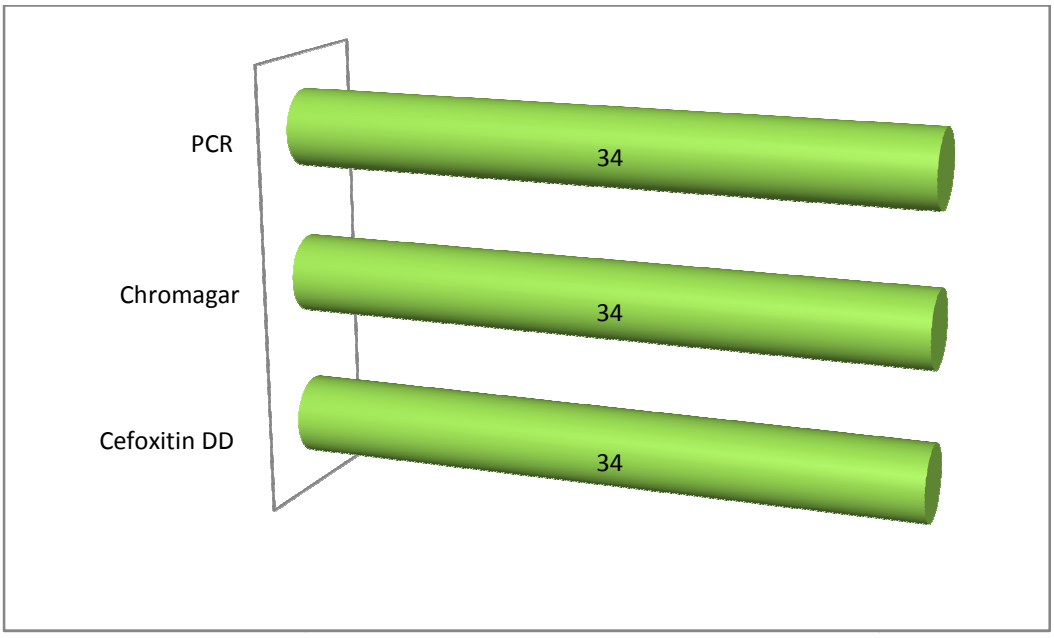
5.4.2. RT-PCR detection of *mecA* gene

Table 3.PCR for *mecA* gene

Total no of *S.aureus* isolates	Method	MRSA
100	Cefoxitin DD	34
	Chromagar	34
	PCR	34

The PCR is considered as the reference method for calculating the sensitivity, specificity, PPV and NPV for the other methods performed for detecting methicillin resistance.

Fig.3 Comparison of Cefoxitin DD method, Chromagar and PCR



5.4.3. Performance characteristics of Cefoxitin DD method & Chromagar

Table 4.Performance characteristics of conventional methods

Method	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Cefoxitin DD test	100	100	100	100
Chromagar	100	100	100	100

The sensitivity, specificity, PPV and NPV of Cefoxitin disc method and Chromagar were 100%, 100%, 100% and 100% respectively. Chromagar was equally efficacious to Cefoxitin disc method for MRSA detection.(Table 4)

5.5. Distribution of MRSA isolates by age and gender

Table 5 shows the distribution of MRSA isolates by age and gender distribution. Most of the MRSA isolates 36% were from ≤ 15 years of age of which all were boys. Three isolates (12%) were from males and two isolates (22.2%) were from females in the 16-30 years age group. In the 31-45 years age group, five isolates (20%) were from males and four isolates (44.4%) were females among MRSA isolates. Six isolates (24%) were from males and two isolates (22.2%) were from females in the 46-60 years age group. Above 61 years, two isolates (8%) were from males

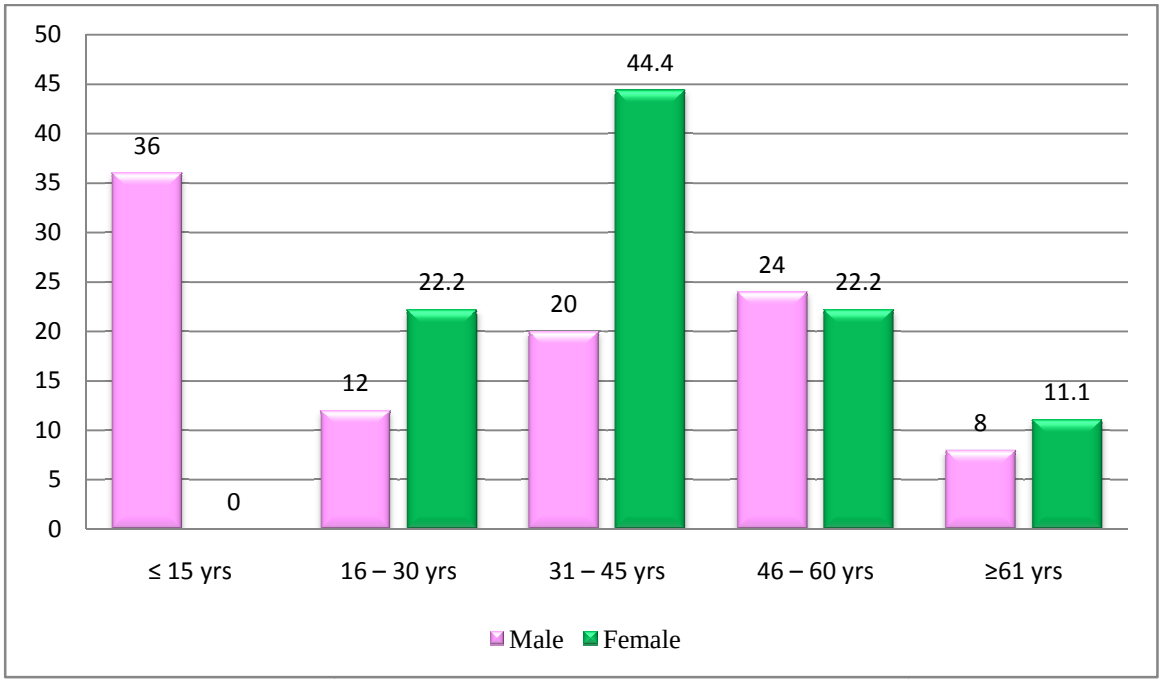
and an isolate (11.1%) was from female. The mean age of male was 30.7 years and that of female was 39.2 years among MRSA isolates and was not significant. ($p > 0.05$) (Figure.4)

Table 5.MRSA isolates by age and gender

Age in years	**MRSA**			
	Male		**Female**	
	No	**%**	**No**	**(%)**
≤ 15	09	36	0	0
16 – 30	03	12	02	22.2
31 – 45	05	20	04	44.4
46 – 60	06	24	02	22.2
≥61	02	08	01	11.1
Total	25	100	09	100
Mean	30.7		39.2	
S.D	23.1		15.6	
't'	1.017			
d.f	32			
p value	> 0.05			

d.f= degrees of freedom

Fig.4. Distribution of MRSA isolates by age and gender



5.6. Categorization of *S.aureus* among outpatients and inpatients

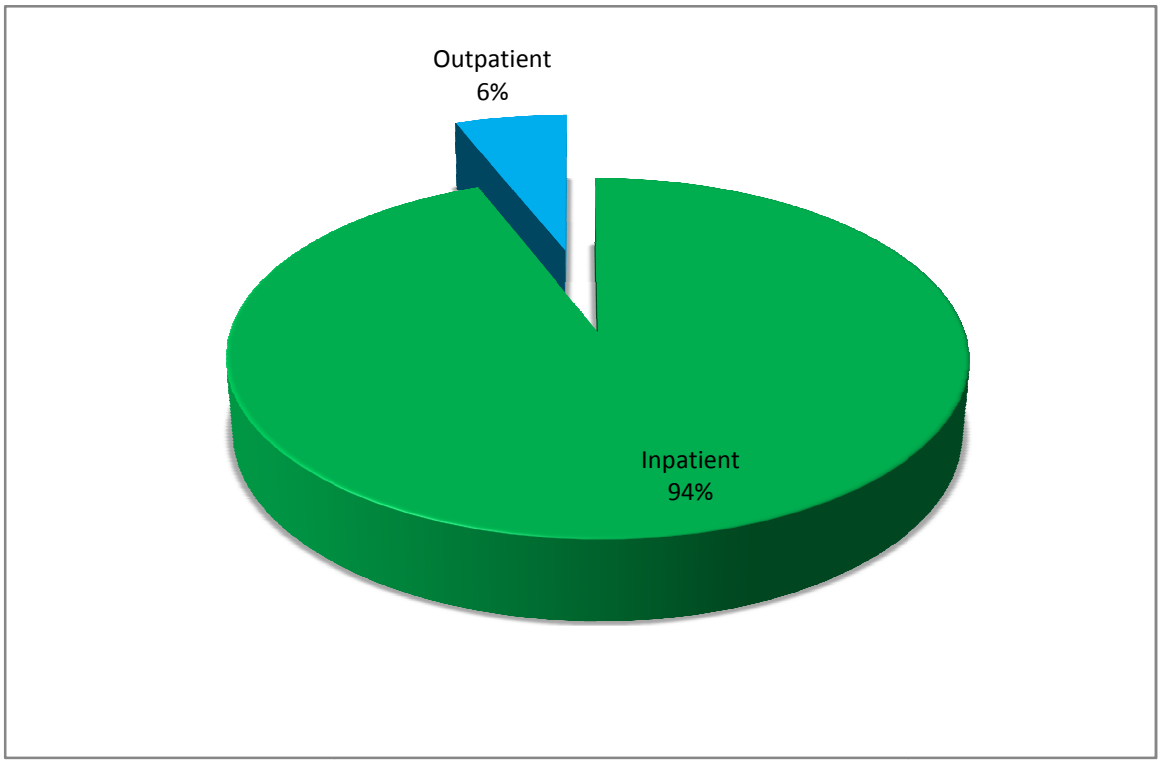
Table 6. Distribution by different categories of patients

Category of the samples	MRSA		MSSA	
	No	%	No	%
Inpatient	32	94.1	62	93.9
Outpatient	2	5.9	4	6.1
Total	34	100	66	100

P value > 0.05

Table.6 shows the distribution of MSSA and MRSA isolates on outpatient and inpatient basis. Majority of the MRSA isolates were in the inpatient group. No significant difference was observed statistically. (fig.5)

Fig.5.MRSA isolates by inpatient and outpatient basis



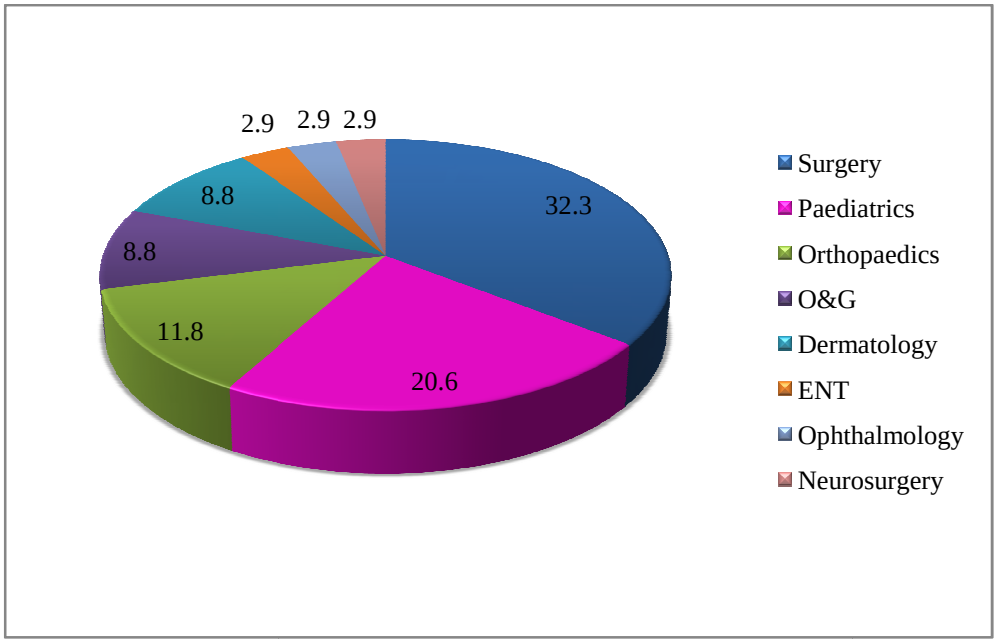
5.7. Distribution of *S.aureus* among samples from various wards

Table 7.MRSA isolation from wards

Ward	MRSA		MSSA	
	No	%	No	%
Surgery	11	32.3	21	31.8
Paediatrics	7	20.6	13	19.7
Orthopaedics	4	11.8	15	22.7
O&G	3	8.8	3	4.5
Dermatology	3	8.8	6	9
ENT	1	2.9	9	13.6
Ophthalmology	1	2.9	-	-
Neurosurgery	1	2.9	-	-
Medicine	-	-	2	3
Total	34	100	66	100

The above table shows the distribution of MRSA samples from various departments of the hospital. Surgery department accounted for the majority of the MRSA isolates i.e 11 (32.3%) of the 34 isolates. Seven isolates were from paediatrics (20.6%), four from orthopaedics (11.8%), three from O&G (8.8%), three from dermatology (8.8%), one from ENT (2.9%), one from ophthalmology (2.9%) and an isolate from neurosurgery (2.9%). (fig.6).

Fig.6 Sample distribution of MRSA from various departments



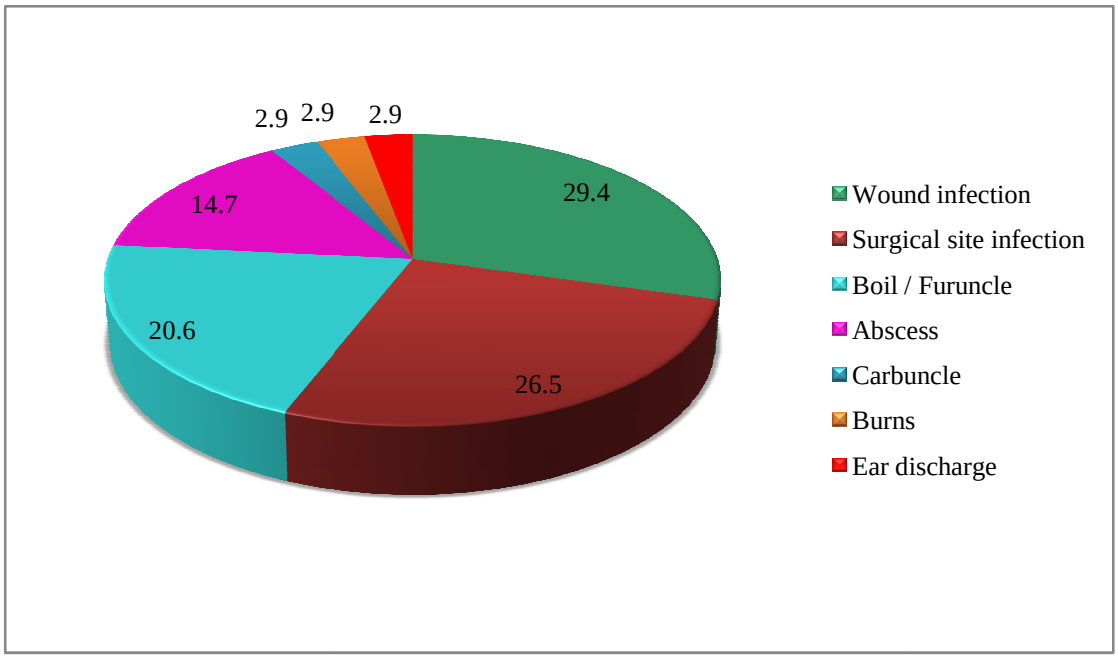
5.8. Association of *S.aureus* with infections

Table 8. MRSA categorization on infection basis

Infections	MRSA		MSSA	
	No	%	No	%
Wound infection	10	29.4	13	19.7
Surgical site infection	9	26.5	26	39.4
Boil / Furuncle	7	20.6	6	9.1
Abscess	5	14.7	9	13.6
Carbuncle	1	2.9	1	1.5
Burns	1	2.9	2	3
Ear discharge	1	2.9	9	13.6
Total	34	100	66	100

Table.8 shows that majority of the MRSA infections are associated with wound infection i.e. 10 (29.4%). Nine isolates from surgical site infection (26.5%), seven from boil/ furuncle (20.6%), five from abscess (14.7%), one from carbuncle (2.9%), one from burns (2.9%) and an isolate from ear discharge (2.9%). (Fig.7)

Fig.7.Association of infections with MRSA



5.9. Duration of hospital stay

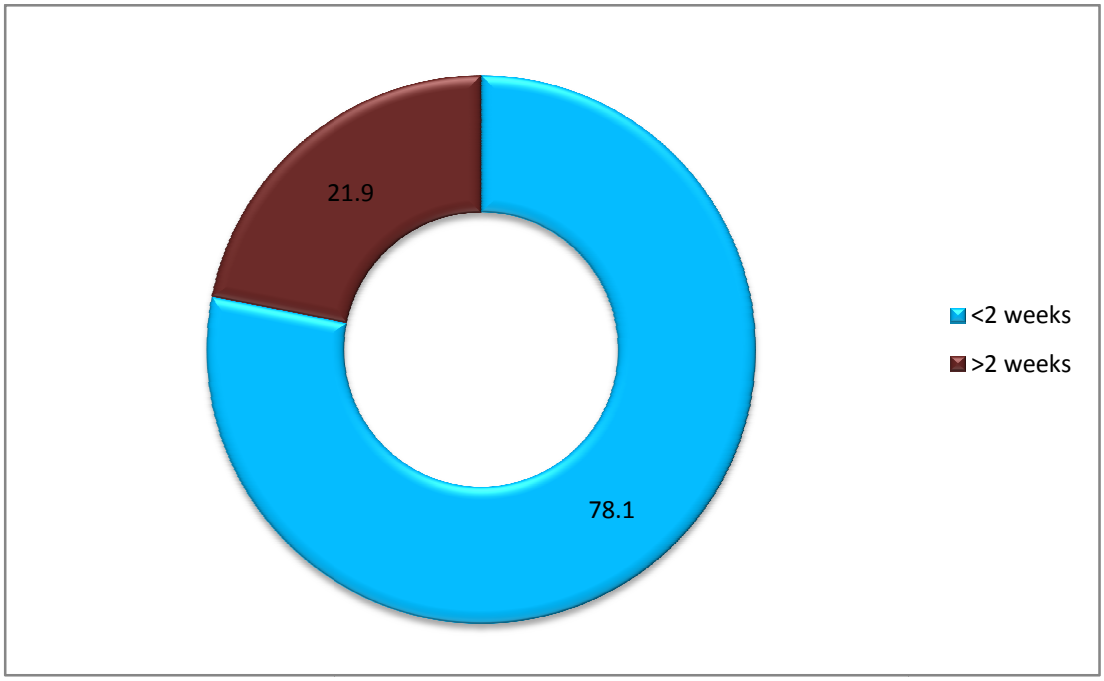
A total of 24 (75%) and eight (25%) of the MRSA isolates were from patients with less than two weeks stay in hospital and more than two weeks respectively. The association of MRSA isolates with the duration of stay in hospital was not significant. (Table.9&fig.8)

Table 9. *S.aureus* isolates by duration of hospital stay

Duration in weeks	MRSA		MSSA	
	No	%	No	%
<2	24	75	49	79
>2	8	25	13	20
Total	32	100	62	100

$p > 0.05$

Fig.8. MRSA isolates by duration of stay at hospital



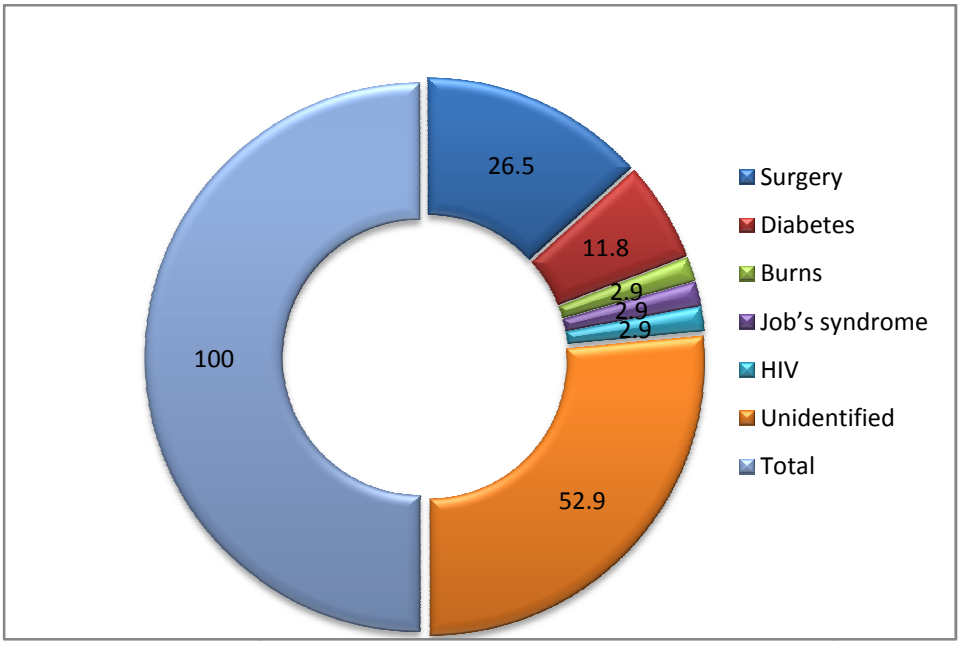
5.10. Association of risk factors with *S.aureus*

Table 10. MRSA and risk factors

Risk factors	MRSA		MSSA	
	No	%	No	%
Surgery	9	26.5	22	33.3
Diabetes	4	11.8	8	12.1
Burns	1	2.9	2	3
Job's syndrome	1	2.9	0	0
HIV	1	2.9	0	0
Unidentified	18	52.9	34	51.5
Total	34	100	66	100

The above table shows the association of risk factors for MRSA isolates. Surgery accounts for 9 (26.5%) of the total 34 isolates. Diabetes constitutes four (11.8%) of the isolates. Burns, HIV and Job's syndrome accounted for each of an MRSA (2.9%) isolate respectively. (fig. 9)

Fig.9 MRSA and risk factors



5.11. Antibiotic sensitivity pattern of *S.aureus*

Table 11. Antibiogram of *S.aureus* isolates

Drug	MSSA			MRSA			p value
	S	I	R	S	I	R	
Penicillin	04	-	62	0	-	34	< 0.05
Cefoxitin	66	-	-	0	-	34	-
Erythromycin	21	40	05	02	11	21	< 0.05
Clindamycin	46	17	03	11	05	18	> 0.05
Gentamicin	44	04	18	07	03	24	> 0.05
Amikacin	52	07	07	13	09	12	< 0.05
Ciprofloxacin	21	16	29	04	08	22	> 0.05
Cotrimoxazole	28	21	17	10	08	16	>0.05
Vancomycin	66	-	0	34	-	0	> 0.05
Teicoplanin	43	23	0	20	13	01	< 0.05
Tigecycline	66	-	0	34	-	0	>0.05
Linezolid	66	-	0	34	-	0	> 0.05

S= Sensitive, I= Intermediate, R= Resistant

Fig.10 and 11 depicts antibiogram of MRSA and MSSA isolates

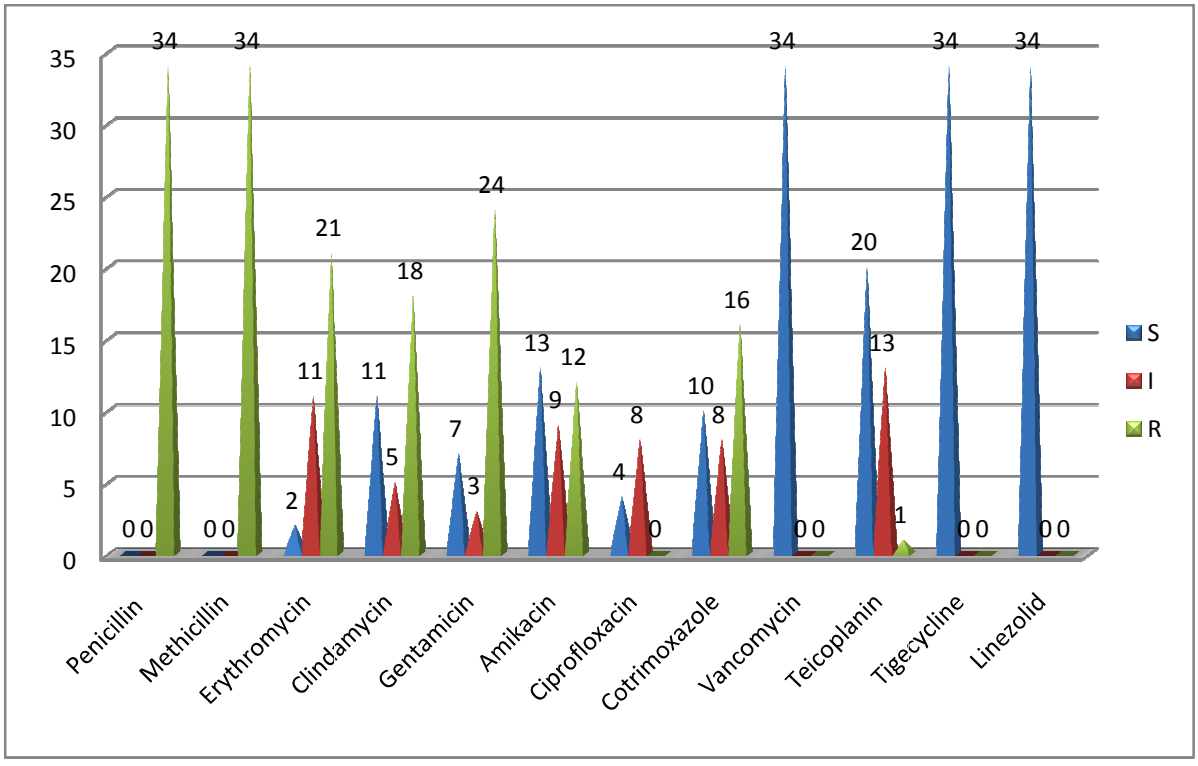
5.11.1. Penicillin

Only four (6.1%) isolates of MSSA were sensitive, while the remaining 62 (93.9%) isolates of MSSA and all the 34 (100%) isolates of MRSA were resistant to Penicillin (10IU). This was found to be statistically significant.

5.11.2. Erythromycin

Among MSSA isolates, 21 (31.8%) were sensitive, 40 (60.6%) were intermediate and five (7.6%) were resistant to Erythromycin (15µg). Two isolates (5.9%) were sensitive, 11 (32.4%) were intermediate and 21 (61.8%) were resistant among MRSA isolates. This was found to be statistically significant.

Fig.10. Antibiogram of MRSA isolates



5.11.3. Clindamycin

Among MSSA isolates, 46 were sensitive (69.7%), 17 were intermediate (25.6%) and three were resistant (4.5%) to Clindamycin (2µg). Eleven isolates were sensitive (32.4%), five were intermediate (14.7%) and 18 isolates were resistant (52.9%) among MRSA isolates. This was not statistically significant.

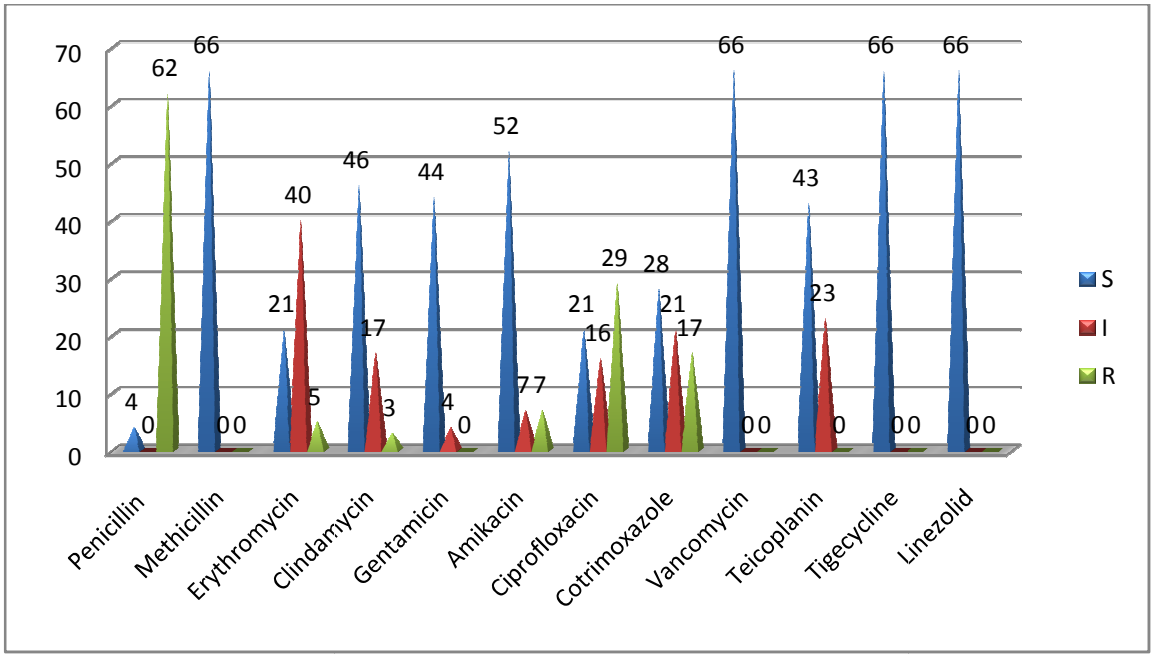
5.11.4. Gentamicin

A total of 44 (66.7%) among MSSA isolates and seven (20.6%) among MRSA isolates were sensitive to Gentamicin (10µg). Four among MSSA (6%) isolates and three among MRSA (8.8%) showed intermediate susceptibility. Resistance was noted among 18 MSSA (27.3%) isolates and 24 (70.6%) MRSA isolates. This was not statistically significant.

5.11.5. Amikacin

A total of 52 (78.8%) were sensitive among MSSA isolates and 13 (38.2%) among MRSA isolates to Amikacin (30µg). A total of seven (9%) were among MSSA and nine (26.5%) among MRSA isolates showed intermediate sensitivity. Resistance was noted among seven (10.6%) MSSA and 12 (35.3%) MRSA isolates. This was found to be statistically significant.

Fig.11. Antibiogram of MSSA isolates



5.11.6. Ciprofloxacin

A total of 21 (31.8%) were sensitive among MSSA isolates and four (11.8%) among MRSA isolates to Ciprofloxacin (5µg). A total of 16 (24.2%) were intermediate among MSSA and eight (23.5%) among MRSA isolates. Resistance was noted among 29 (44%) MSSA isolates and 22 (64.7%) MRSA isolates. This was found to be statistically significant.

5.11.7. Co-trimoxazole

A total of 28 (42.4%) were sensitive among MSSA isolates and 10 (29.4%) among MRSA isolates to Co-trimoxazole (1.25/23.75 µg). Intermediate susceptibility was shown by 21 (31.8%) among MSSA and eight (23.5%) among MRSA isolates. Resistance was noted among 17

(25.6%) MSSA isolates and 16 (47.1%) MRSA isolates. This was not statistically significant.

5.11.8. Vancomycin

All the isolates were sensitive (100%) to Vancomycin (30µg) and none of them were resistant to the drug among MRSA and MSSA isolates. All the isolates were sensitive by Vancomycin agar screen method also.

5.11.9. Teicoplanin

A total of 43 (65.2%) were sensitive among MSSA isolates and 20 (58.8%) among MRSA isolates to Teicoplanin (30µg). Twenty three (34.8%) were intermediate sensitive among MSSA and 13 (38.3%) among MRSA isolates. Resistance was not noted among 66 MSSA isolates, but an isolate was resistant (3%) among MRSA. This was found to be statistically significant.

5.11.10. Tigecycline

All the isolates were sensitive (100%) to Tigecycline (15µg) and none of them were resistant to the drug among MRSA and MSSA isolates.

5.11.11. Linezolid

All the MRSA and MSSA isolates were sensitive (100%) to Linezolid (30µg) and no resistance was noted.

5.12. Inducible Clindamycin resistance

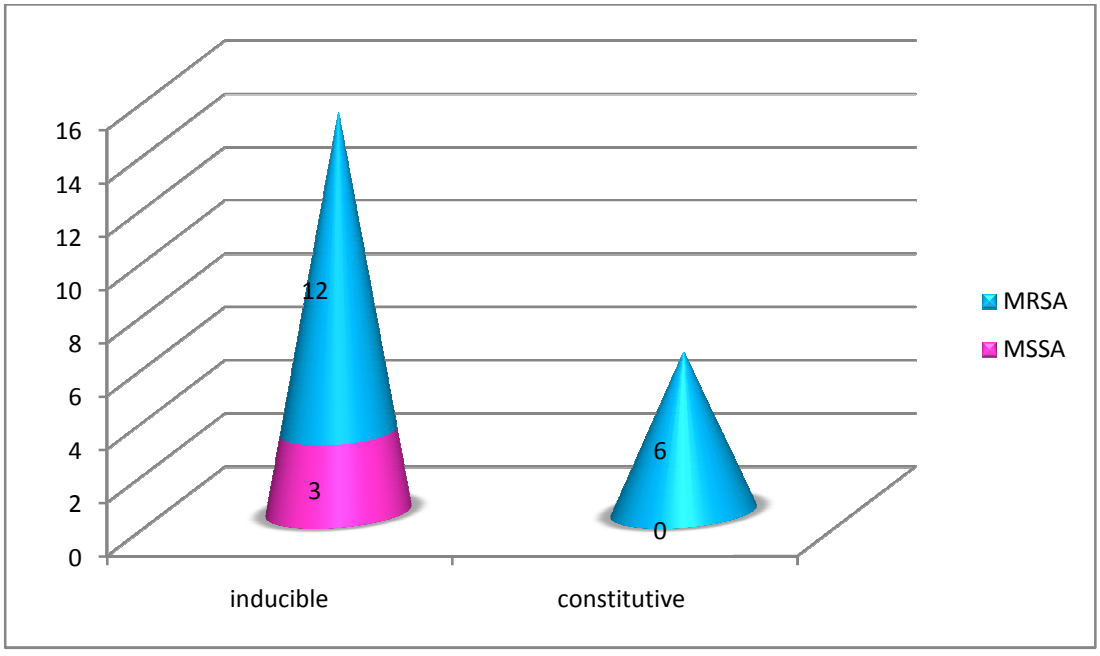
Table.12. D-Test

	Inducible Resistance	**Constitutive resistance**	**Total resistance**
MSSA	3	0	3
MRSA	12	6	18
Total	15	6	21

$p > 0.05$

Table 12 shows, inducible Clindamycin resistance among *S.aureus* isolates. Among the MSSA, three isolates showed inducible Clindamycin resistance. But among MRSA, 12 isolates showed inducible Clindamycin resistance. No significant difference was observed between MSSA and MRSA isolates. (fig.12)

Fig.12 D - Test



5.13. Multidrug resistance among MRSA isolates

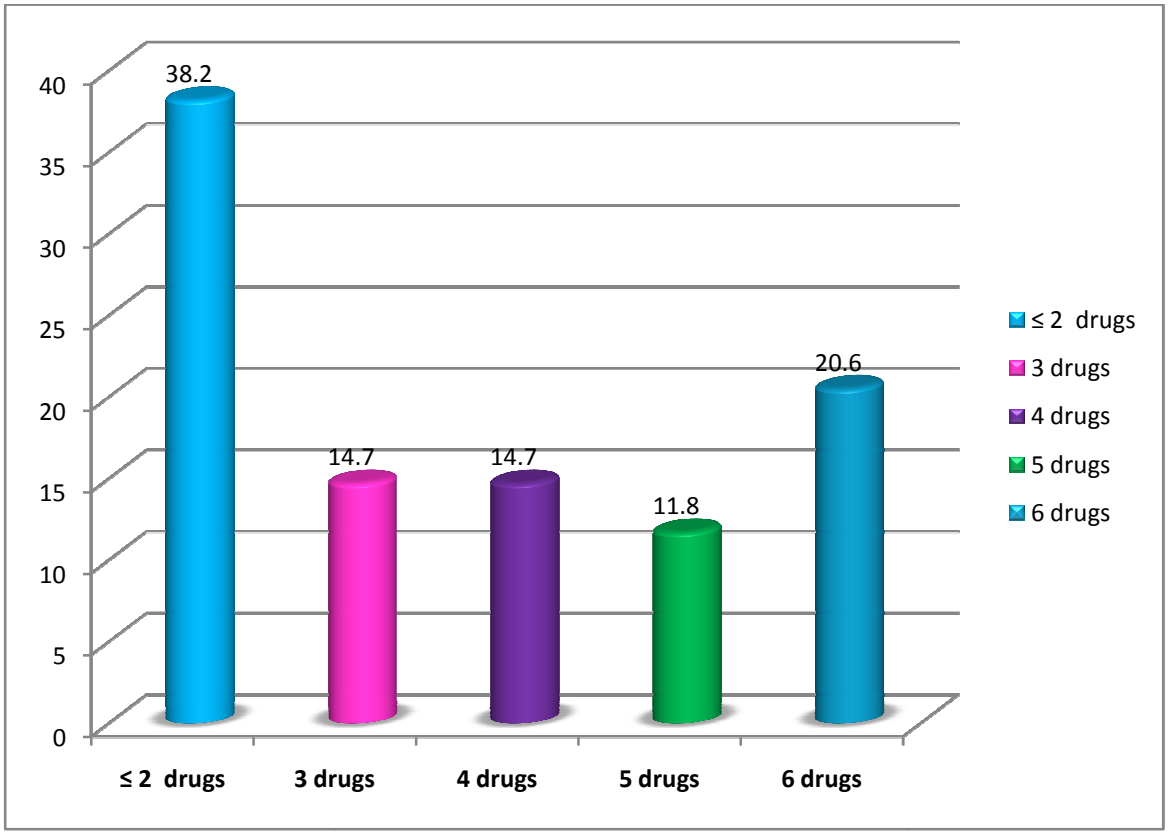
An isolate of MRSA is considered to be multidrug resistant if resistance was noted among ≥ 3 drugs (excluding Penicillin and Cefoxitin) in this study.

Table 13. Multidrug resistance

No. of drugs	MRSA isolate	%
≤ 2	13	38.2
3	05	14.7
4	05	14.7
5	04	11.8
6	07	20.6
Total	34	100

Five MRSA isolates (14.7%) were resistant for ≥ three drugs, five isolates (14.7%) for four drugs, and four isolates (11.8%) for five drugs. Seven isolates (20.6%) were resistant for six drugs- Erythromycin, Clindamycin, Gentamicin, Amikacin, Ciprofloxacin and Co-trimoxazole in the present study. (Fig.13)

Fig.13 Multidrug resistance of MRSA



Discussion

6. DISCUSSION

The early detection of Methicillin resistance is of prime importance in the prognosis of *S.aureus* infections. The identification of MRSA is determined by the method performed, which can vary in sensitivity and specificity. Factors that impact culture-based detection of MRSA include incubation time, temperature and accurate interpretation. Enhanced detection along with the cost effectiveness has a key role in clinical laboratories with limited resources. In this study, various methods are evaluated for detecting Methicillin resistance and PCR is considered the "gold standard" for detecting the *mecA* gene.

6.1. Prevalence

Prevalence of MRSA in the present study was 34% among the pus samples. This is comparable with the study conducted by Buzaid et al,[69] from Libya where the prevalence was found to be 31%. The study by Srinivasan et al,[44] found MRSA prevalence to be 33% which is similar to the present study. But the study by Sharma et al,[42] from Himachal Pradesh found the prevalence rate to be 23.71%.

The study by Vidhani et al,[70] found the prevalence among the high risk patients as high as 51.6%. Majumer et al,[71] from Assam, observed 52.9% prevalence of MRSA. But the study from Eritrea[72] had only 9% prevalence of MRSA.

6.2. Distribution of MRSA isolates

In this study, majority of the MRSA isolates were from males (25%) while the remaining were females (9%). This is similar to the study by Thangavel et al,[41] where males 30% constituted most of the MRSA isolates while 10% was from females. On contrary, the study from Doon valley hospitals,[42] had showed that most of the MRSA isolates were from females 60.86% while males had 39.13% only. The study by Buzaid et al,[69] found no significant difference between males 28 (31.8%) and females 34 (30.4%) among MRSA isolates.

Majority of the MRSA isolates was from the surgery department 32.3%, followed by paediatrics 20.6%, orthopaedics 11.8%, and dermatology 8.8% in the present study. The study by Arora et al,[26] too had found highest prevalence from surgical units (54.8%). Surgery was definitely an associated factor in MRSA infections as those patients are bound to take antimicrobials for long duration. The highest percentage of isolates were from Intensive Care Units (34%) in the study by Sadaka et al[73]. Sarma et al,[54] had found that MRSA was associated with 34% of infections in orthopaedics and 18% in surgical units but 1% in medical units. The study by from Amristar[26] showed that, orthopedic patients accounted for the maximum number of MRSA strains (27.8%).

In this study, almost most of the MRSA were isolated from wound infection 29.4% while surgical site infections constituted 26.5%. The

remaining isolates were from boil/furuncle 20.6%, abscess 14.7% and burns 2.9%. This is comparable to the study by Terry Ali et al,[53] where wound infection (21.4%), isolated majority of the MRSA isolates. The study from PIMS,[44] found a high prevalence from post operative surgical infections (80%). Frazee et al,[74] found that MRSA,was strongly associated with infection type- furuncle.

6.3. Risk factors

Most of the MRSA isolates were from patients who had undergone surgery 26.5%, while 11.8% isolates with diabetes, 2.9% with burns, 2.9% with HIV and 2.9% with Job's syndrome. Surgical site infections, both superficial and deep, could be caused by MRSA. Repeated surgeries and hence prolonged stayal, paves way for MRSA infections. Among diabetics, the decrease in the neutrophil activity plays a predominant role for MRSA infection. But the study from New Delhi[75] found a 51.6% association with high risk patients like burns. This was attributed to the longer stay in hospital and the usage of multiple antibiotics.

No significant association was found between MRSA and MSSA isolates for treatment on outpatient and inpatient basis in the present study. The inpatients had the highest rate (15.6%) compared to the outpatients (3.8%) in the study from JIPMER.[43]

In this study, duration of stay at hospital was not a risk factor for MRSA infection. Similarly, the study from Northeastern India[54] had

found that duration of hospital stay had no significant association with MRSA infection. But Mehta et al,[51] observed significant association of MRSA and hospital stay of more than 15 days.

6.4. Laboratory diagnosis

Methicillin detection in laboratories is based upon a wide array of tests like conventional, Chromagar and molecular methods. The detection of *mecA* gene by PCR has stood best of time and is considered the "gold standard" technique for Methicillin resistance. PCR requires lesser time to perform and the results are rapid in few hours. But its limitations are: needs skills to perform, high cost and chance for cross contamination.

6.4.1. Cefoxitin DD method

In the present study, Cefoxitin DD method identified 34 MRSA isolates which were confirmed by PCR and had sensitivity, specificity, PPV and NPV of 100% respectively. The study by Rao et al,[61] and Gupta et al,[56] too found that all the MRSA isolates were identified by Cefoxitin DD method and is found to have 100% sensitivity and specificity.

Hujer at al[45] found the sensitivity and specificity of 97% and 97.4% for Cefoxitin DD test. But the study from Egypt,[73] found that the sensitivity and specifcity of Cefoxitin DD method to be 98.6% and 72.4% only, while the PPV and NPV were 89.7% and 95.5% respectively.

CLSI recommends Cefoxitin DD method for detecting Methicillin resistance in clinical laboratories. The method is easy to be carried out, no

special training is required and interpreting the zone sizes too is also not so difficult. But Cefoxitin DD method requires another 24 hours to detect Methicillin isolates after isolation of the organism and is unable to pick up the non-*mecA* mediated Methicillin resistance.

6.4.2. Chromagar

In this study, Chromagar identified 34 (100%) isolates from the pus samples at 24 hours of incubation itself and no isolates showed growth after 48 hours of incubation. The sensitivity, specificity, PPV and NPV were 100% respectively for Chromagar. The study from Japan[76] found the same results where the Chromagar identified all the MRSA isolates and 100% sensitivity and specificity was determined. Van Hoecke et al,[77] found that Chromagar showed growth of all MRSA isolates at 24 hours itself, thus avoiding further incubation.

This is in contrast with the study by Kaur et al,[78] where Chromagar misidentified 11 MSSA isolates as MRSA and had a sensitivity of 77.27% and specificity of 79.25% only. In the study by Thangaavel et al[41] too, Chromagar identified 7 false positives. Paul et al,[79] evaluated Chromagar with PCR for MRSA detection and found that at 24 hours the sensitivity and specificity were 85.4% and 99.4% which increased to 87.7% and 100% at 48 hours of incubation.

Chromogenic medium had emerged as a newer method for selective and rapid detection of MRSA isolates. This method has the

added advantage of interpreting the result easily by observing the coloured colonies. They are developed with higher sensitivity and specificity too. It is ideal to use Chromagar in clinical samples so that detection of MRSA isolates can be earlier than the conventional methods. The disadvantage is the prolonged incubation if no growth occurs.

5. Antibiotic resistance

6.5.1. Penicillin

Only four (6.1%) isolates were sensitive to Penicillin among the MSSA isolates in the present study. This is comparable to the study by Vidhani et al,[70] where six (5.5%) isolates were sensitive to Penicillin among MSSA isolates. In the study bySarma et al,[54] 18% of the MSSA isolates were sensitive to Penicillin.

6.5.2. Erythromycin

Among the Macrolides, Erythromycin resistance seems to be on rise among MRSA isolates. In the present study, resistance was noted among 5 (7.6%) of the MSSA isolates while it was 21 (61.8%) among MRSA isolates. Arora et al,[26] found that 61.7% of MRSA isolates were resistant to this drug. Frazee et al,[74] and Rao et al,[61] found 56.8% and 45-48% resistance among MRSA isolates. This is in contrast to the study from Coimbatore,[40] where the Erythromycin resistance was 20.5% only. The role of Erythromycin as an alternative among MRSA infections is uncertain because of higher resistance.

6.5.3. Inducible clindamycin resistance

The present study showed the inducible Clindamycin resistance as 12% and the constitutive resistance was 6% among MRSA isolates while the inducible Clindamycin resistance was only 3% among MSSA isolates. The D-test had identified 12 isolates which would had been considered sensitive to Clindamycin leading to inappropriate treatment. This is in comparable to the study by Sireesha et al[80] where the inducible Clindamycin resistance was 18%. Deotale et al,[81]found 27.6% inducible Clindamycin resistance among MRSA isolates. This is in contrast to the studies by Ajantha et al,[82]where 74% of MRSA were positive for D-test.

Constitutive resistance was observed in 6% of MRSA isolates in the present study. But the study by Angel et al[83] had found that none of the MRSA isolates exhibited it. Detection of inducible Clindamycin resistance has a pivotal role in the clinical laboratories as it helps to avoid therapeutic failure.

6.5.4. Gentamicin

Gentamicin resistance was 18 (27.3%) among MSSA isolates and 24 (70.6%) among MRSA isolates. Terry Ali et al[53] had found 56.5% resistance and the study from Manglore[61] found 40-50% of the MRSA isolates were resistant to Gentamicin. A 100% resistance was observed among the MRSA isolates in the study from Assam.[71] But the study by Rajaduraipandi et al[40] had reported 20.5% to Gentamicin among MRSA

isolates. Among Aminoglycosides, the role of Gentamicin for MRSA infections can be ruled out, as it is frequently administered for Gram negative infections.

6.5.5. Amikacin

In this study, 7 (10.6%) resistance among MSSA isolates and 12 (35.3%) among MRSA isolates was noted for Amikacin. This is similar to the study from Amristar,[26] where the resistance to Amikacin was 12 (8.9%) among MSSA isolates and 43 (37.4%) among MRSA isolates. This is in opposition to the study by Mullah et al,[36] where the Amikacin resistance was found to be 52.6%. But a recent study from Iran,[84] had showed only 13.8% resistance. Amikacin still remains effective for MRSA infections.

6.5.6. Ciprofloxacin

Resistance to Ciprofloxacin is considered to be surrogate marker for MRSA infections. In this study, Ciprofloxacin resistance was found to be 64.7% among MRSA isolates. The study by Rao et al[61] found 53-56% resistance for Ciprofloxacin among MRSA isolates. The study by Pai et al[70] found only 31.8% resistance among MRSA isolates. Quershi et al,[52]found higher resistance among MRSA isolates (90%) to Ciprofloxacin.

6.5.7. Vancomycin

In the present study, all the isolates were sensitive to Vancomycin. This is similar to the studies by Terry Alli et al[53] and Arora et al,[26] where Vancomycin resistance was not found among MRSA isolates. This is in contrast to the study by Assadullah et al,[33] where 4 (3.3%) isolates had low level resistance to vancomycin among MRSA isolates. Vancomycin is the reserve drug for serious MRSA infections. DD method for Vancomycin has to be confirmed with agar screen, dilution or molecular methods as per CLSI guidelines.[67]

6.5.8. Teic oplanin

Resistance to Glycopeptides like Teicoplanin is of concern today, since only a handful of drugs are available for MRSA infections. Teicoplanin resistance was noted in an isolate of MRSA in the present study. The studies from India,[34,54]had found 100% sensitivity among MRSA isolates to Teicoplanin.

6.5.9. Linezolid

All the isolates were susceptible (100%) to Linezolid. The study by Srinivasan et al[44] found that all the MRSA isolates were susceptible to the drug. Rajaduraipandi et al[40], found that 97.6% of the MRSA isolates were sensitive to this drug. Linezolid remains as an effective drug for MRSA infections.

6.6. Multidrug resistance

In this study, seven (20.5%) isolates had resistance to six drugs tested while four (11.7%) had resistance to five of the drugs tested. Multidrug resistance was noted among 21% of the MRSA isolates. This is similar to the study by Majumer et al,[71] where 23.2% multidrug resistance was reported among MRSA isolates. Zeinalli et al[84] too confirmed the multidrug resistance among 87 MRSA isolates.

Multidrug resistance was observed more among MRSA isolates than MSSA isolates. In the present study, multidrug resistance was noted for the following drugs like Erythromycin, Clindamycin, Gentamicin, Amikacin, Ciprofloxacin and Co-trimoxazole. These are considered as the first line treatment for MRSA infections and their resistance is of major concern as treatment goes in favour of glycopeptides, which are the reserve drugs and a bit costly too. This can give rise to unexpected outbreaks in hospital.

The present study revealed that the Chromagar was highly sensitive and specific for detecting Methicillin resistance. It is equally efficacious in detecting Methicillin resistance to Cefoxitin DD method. The colouration of the colonies makes visual interpretation so easy. In clinical laboratories, with lacking technical facilities, disc based tests consume ample time in processing. Chromagar attempts to reduce this and so this

method can reliably be used as an alternative where *mecA* gene detection is not feasible.

The marked difference between the antibiogram of MRSA and MSSA isolates makes difficult to interpret routine antibiotic susceptibility testing of *S.aureus*. The multidrug resistance of MRSA isolates is of alarming problem. Difficulty in deciding the initial treatment exists for *S.aureus* and so it is wise to perform antibiotic sensitivity testing for all *S.aureus* isolates before initiating treatment. The effort for manufacturing newer antibiotic for Gram positive infections remains uncertain and only fewer antimicrobials remains as alternatives. An integrated awareness program, good hand washing techniques along with frequent epidemiological studies and effective control measures are the goals for MRSA elimination in hospitals in the future.

Summary

7. SUMMARY

This study was undertaken at Tirunelveli Medical College, Tirunelveli for a period of one year from 100 *S.aureus* isolates isolated from pus samples. These isolates were assessed for the antibiotic sensitivity pattern and Methicillin resistance by Cefoxitin DD method, Chromagar and PCR and the various risk factors were analysed.

- A total of 62 isolates of *S.aureus*, were from males and the remaining 38 were from females.
- Cefoxitin DD method identified 34 MRSA isolates by measuring the zone size of ≤ 21 mm in diameter.
- The sensitivity and specificity of Cefoxitin DD method was 100 % while PPV and NPV were 100% respectively.
- Chromagar showed growth of bluish green colonies for the same 34 isolates.
- The sensitivity and specificity of Chromagar was 100 % while PPV and NPV were 100% respectively.
- Real time PCR detected *mecA* gene in the 34 isolates which is considered the 'gold standard' in this study.
- The prevalence of the MRSA isolates from pus samples was 34%.
- A total of 25% of the MRSA isolates were from males and 9% were from females.

- Mean age of the MRSA isolates from male was 30.7 years and that of female was 39.2 years which was not considered significant.
- Most of the MRSA isolates 9 (36%) were from ≤ 15 years of age of which all were boys.
- No significant association was found between inpatients and outpatients.
- Surgery accounted for 11 (32.3%) of the MRSA isolates while seven (20.6%) from paediatrics and four (11.8 %) were from orthopaedics.
- Wound infections constituted majority 10 (29.4%) of the infections by MRSA isolates followed by surgical site infections nine (26.5%) and boil/ furuncle seven (17.6%).
- Duration of stay at hospital for more than two weeks had no significant association with the MRSA infection.
- The associated risk factors for MRSA infection were surgery (26.5%), diabetes (11.8%), burns, HIV and Job's syndrome each one (2.9%) respectively.
- Only four (6.1%) of the MSSA isolates were sensitive to Penicillin.
- A total of five (7.6%) of the MSSA isolates and 21 (61.8%) of the MRSA isolates were resistant to Erythromycin.

- Resistance to Gentamicin were noted with 18 (27.3%) of MSSA isolates while it is 24 (70.6%) of the MSSA isolates.
- A total of 13 (38.2%) of the MRSA and 52 (78.8%) of the MSSA isolates were sensitive to Amikacin.
- Resistance was shown by 29 (44%) of the MSSA isolates and 22 (64.7%) of the MRSA isolates to Ciprofloxacin.
- A total of 22 (42.4%) were susceptible among MSSA and 10 (29.4%) among MRSA isolates to Co-trimoxazole.
- All the isolates both MSSA and MRSA were sensitive to Vancomycin, Tigecycline and Linezolid.
- Resistance was shown by an MRSA isolate to the Glycopeptide, Teicoplanin.
- Inducible Clindamycin resistance was 12% and constitutive resistance was 6% among MRSA isolates while 3% of the MSSA isolates were positive for D-test.
- Multidrug resistance was noted among 21% of the MRSA isolates to the commonly used drugs like Erythromycin, Ciprofloxacin, Clindamycin, Gentamicin, Amikacin And Co-trimoxazole.

Conclusion

8. CONCLUSION

- This study highlights the prevalence of MRSA among clinical samples conditioning the patient for prolonged treatment and associated risk factors adds to the problem.
- Antibiogram of Methicillin sensitive and resistant isolates differs and susceptibility testing is mandatory for clinical isolates of *S.aureus* before initiation of treatment as few antibiotics exist for serious MRSA infections.
- DD methods are easy to perform and cost effective in Methicillin resistance detection and is the standard procedure which tends to be followed in many laboratories.
- Chromagar is to be considered as a diagnostic tool for Methicillin resistance because of its rapid and easy interpretation, high sensitivity and specificity and lack of skill in performing the method.
- Real time PCR continues to remain as “gold standard” for *mecA* gene detection because of its high specificity.
- An approach to eliminate MRSA in the hospitals and community needs to be integrated by creating awareness among people and good hygienic practices and effective barrier precautions are to be adapted to prevent further transmission.

EVALUATION OF CHROMAGAR AND PCR FOR DETECTION OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) FROM CLINICAL ISOLATES

ANNEXURE -III MASTER CHART

SL.NO	Age	IP/OP	Sex	Ward	Diagnosis	Duration	Risk factors	Peni cillin	Methi cillin	Erythro mycin	Clinda mycin	Genta mycin	Amikacin	Cipro floxacin	Sep tran	Van comycin	Teico planin	Line zolid	Tige cyline	CHRO Magar	PCR	Ct Value
1	26	IP	M	surgery	wound infection	?2 weeks	-	R	R	R	S	I	I	S	I	S	I	S	S	+	+	23.17
2	25D	IP	M	surgery	wound infection	?2 weeks	-	R	R	I	S	R	I	I	I	S	I	S	S	+	+	15.21
3	8	IP	M	paediatrics	wound infection	?2 weeks	-	R	S	I	S	S	S	S	R	S	S	S	S	-		
4	11	IP	M	paediatrics	wound infection	?2 weeks	-	R	S	I	S	R	I	R	R	S	I	S	S	-		
5	37	IP	F	surgery	abscess	?2 weeks	-	R	R	R	R(D test)	I	S	R	S	S	S	S	S	+	+	16.14
6	52	IP	F	surgery	wound infection	?2 weeks	diabetes	R	S	S	S	S	S	S	I	S	S	S	S	-		
7	45	IP	F	surgery	wound infection	?2 weeks	diabetes	R	S	R	R(D test)	R	R	R	R	S	I	S	S	-		
8	36	IP	F	ENT	ear discharge	?2 weeks	-	R	S	I	S	R	S	R	R	S	S	S	S	-		
9	15	IP	M	surgery	surgical site inf	?2 weeks	surgery	R	S	S	S	S	S	S	S	S	S	S	S	-		
10	40	IP	M	ortho	wound infection	?2 weeks	-	R	R	I	R	S	S	S	S	S	S	S	S	+	+	14.13
11	29	IP	M	surgery	surgical site inf	?2 weeks	-	R	S	I	I	S	R	S	S	S	S	S	S	-		
12	67	IP	M	surgery	wound infection	?2 weeks	-	R	S	S	I	S	S	S	S	S	S	S	S	-		
13	35	IP	F	surgery	surgical site inf	?2 weeks	surgery	R	R	R	R(D test)	S	S	R	S	S	S	S	S	+	+	14.35
14	17	IP	M	surgery	abscess	?2 weeks	-	R	S	I	I	S	S	S	I	S	I	S	S	-		
15	12	OP	M	ENT	ear discharge	-	-	R	S	I	I	S	S	S	S	S	I	S	S	-		
16	42	IP	M	surgery	surgical site inf	?2 weeks	surgery	R	S	I	I	S	S	S	I	S	S	S	S	-		
17	38	IP	M	surgery	abscess	?2 weeks	-	R	R	I	R	S	I	I	S	S	R	S	S	+	+	13.22
18	8	IP	F	paediatrics	boil	?2 weeks	-	R	S	S	S	S	S	S	S	S	S	S	S	-		
19	60	IP	M	surgery	surgical site inf	?2 weeks	diabetes	R	S	R	R(D test)	R	R	R	I	S	R	S	S	-		
20	31	IP	M	ortho	wound infection	?2 weeks	-	R	S	R	R(D test)	R	R	R	I	S	I	S	S	-		
21	46	IP	M	surgery	surgical site inf	?2 weeks	surgery	R	R	I	I	R	R	I	S	S	S	S	S	+	+	14.78
22	32	IP	F	surgery	burns	?2 weeks	burns	R	R	S	S	S	S	S	S	S	I	S	S	-		
23	6D	IP	F	paediatrics	wound infection	?2 weeks	-	R	S	I	I	S	S	S	S	S	I	S	S	-		
24	50D	IP	M	paediatrics	boil	?2 weeks	-	R	R	I	I	S	S	S	S	S	S	S	S	+	+	14
25	27	IP	M	ortho	surgical site inf	?2 weeks	surgery	R	S	S	I	S	S	I	S	S	S	S	S	-		
26	60	IP	M	surgery	wound infection	?2 weeks	diabetes	R	R	R	R(D test)	R	R	R	R	S	I	S	S	+	+	19.65
27	72	IP	M	ortho	surgical site inf	?2 weeks	diabetes	R	S	S	S	S	S	S	S	S	S	S	S	-		
28	49	IP	M	ortho	surgical site inf	?2 weeks	surgery	R	S	I	S	R	R	R	I	S	I	S	S	-		
29	3	IP	M	paediatrics	surgical site inf	?2 weeks	surgery	R	R	R	R(D test)	R	R	R	R	S	S	S	S	+	+	15.57
30	29	IP	M	ortho	surgical site inf	?2 weeks	surgery	R	S	I	S	S	S	S	I	S	S	S	S	-		
31	75	IP	M	surgery	surgical site inf	?2 weeks	surgery	R	S	S	S	S	S	R	S	S	I	S	S	-		
32	57	IP	M	skin	boil	?2 weeks	diabetes	R	R	S	S	R	S	R	I	S	S	S	S	+	+	11.89
33	32	IP	F	surgery	surgical site inf	?2 weeks	surgery	R	S	I	S	R	I	R	R	S	S	S	S	-		

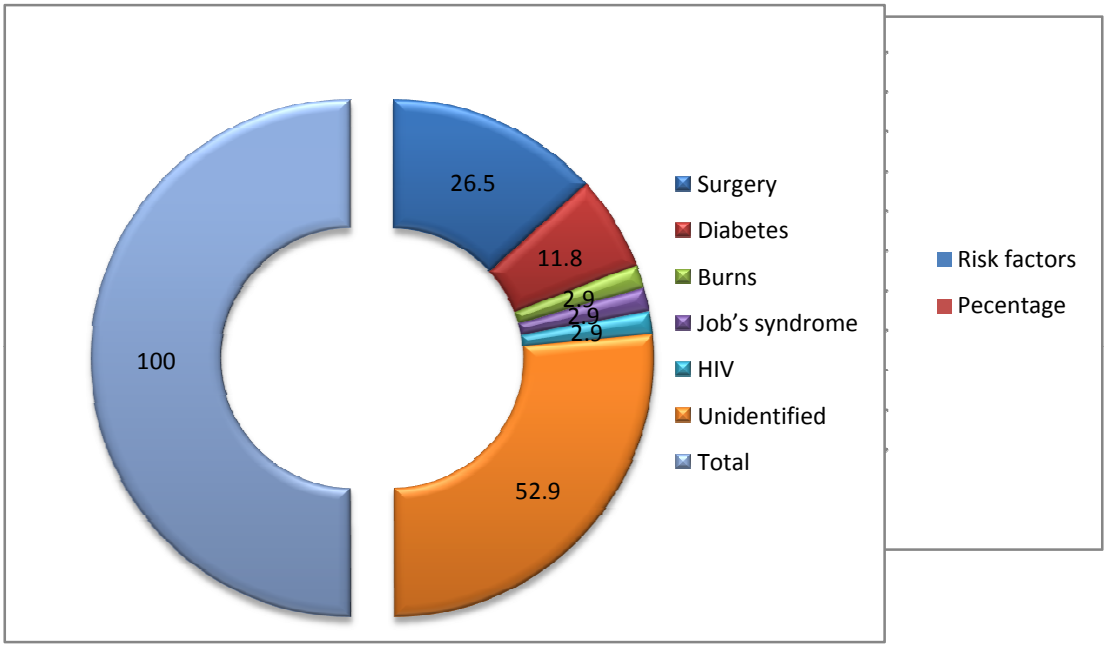
34	48	IP	M	skin	boil	?2 weeks	-	R	S	I	S	R	I	R	I	S	S	S	S	-		
35	8	IP	F	skin	boil	?2 weeks	b syndron	R	R	I	I	R	I	R	R	S	I	S	S	-		
36	59	OP	F	surgery	abscess	-	-	R	S	I	S	S	S	R	S	S	S	S	S	-		
37	4	IP	M	paediatrics	wound infection	?2 weeks	-	R	R	I	S	S	I	R	R	S	I	S	S	+	+	16.35
38	36	IP	M	ortho	surgical site inf	?2 weeks	surgery	R	R	R	R(D test)	R	R	R	R	S	I	S	S	+	+	14.7
39	33	IP	M	ortho	surgical site inf	?2 weeks	surgery	R	S	I	S	R	S	I	S	S	S	S	S	-		
40	23	IP	F	OG	surgical site inf	?2 weeks	surgery	S	S	S	S	S	S	S	S	S	S	S	S	-		
41	15D	IP	F	paediatrics	wound infection	?2 weeks	-	R	S	I	S	R	I	I	I	S	S	S	S	-		
42	45	IP	M	surgery	carbuncle	?2 weeks	diabetes	R	S	S	S	R	S	I	S	S	S	S	S	-		
43	45	IP	M	surgery	burns	?2 weeks	burns	R	S	I	S	I	S	R	I	S	S	S	S	-		
44	11	IP	F	paediatrics	abscess	?2 weeks	-	R	S	S	I	R	S	R	I	S	S	S	S	-		
45	2	IP	F	paediatrics	boil	?2 weeks	-	R	S	S	S	S	S	S	S	S	S	S	S	-		
46	74	IP	F	ENT	ear discharge	?2 weeks	-	R	S	I	S	R	S	R	S	S	S	S	S	-		
47	46	IP	F	skin	boil	?2 weeks	-	R	R	R	R(D test)	R	R	R	R	S	S	S	S	+	+	22.44
48	41	IP	M	ortho	surgical site inf	?2 weeks	surgery	R	S	S	S	S	S	S	I	S	S	S	S	-		
49	20	IP	F	OG	surgical site inf	?2 weeks	surgery	R	R	R	R(D test)	R	I	R	R	S	S	S	S	+	+	13.27
50	8	IP	F	skin	boil	?2 weeks	-	R	S	I	S	R	S	R	I	S	S	S	S	-		
51	70	IP	F	ophthal	abscess	?2 weeks	-	R	R	R	R(D test)	R	R	R	I	S	S	S	S	+	+	16.35
52	15D	IP	F	paediatrics	abscess	?2 weeks	-	R	S	I	S	S	S	R	S	S	I	S	S	-		
53	3	OP	F	paediatrics	abscess	-	-	R	S	S	S	I	S	I	R	S	S	S	S	-		
54	55	IP	M	ortho	surgical site inf	?2 weeks	surgery	R	R	R	R(D test)	R	S	I	R	S	S	S	S	+	+	12.93
55	65	IP	M	neurosurgery	abscess	?2 weeks	-	R	R	R	R(D test)	R	I	R	R	S	S	S	S	+	+	17.3
56	12	IP	M	ortho	surgical site inf	?2 weeks	surgery	R	S	I	S	S	S	R	I	S	S	S	S	-		
57	19	IP	M	surgery	abscess	?2 weeks	-	R	R	I	S	R	S	R	R	S	S	S	S	+	+	19.36
58	11	IP	M	paediatrics	boil	?2 weeks	-	R	R	I	S	S	S	R	R	S	S	S	S	+	+	14.04
59	15	IP	M	surgery	wound infection	?2 weeks	-	R	S	I	S	S	S	R	S	S	I	S	S	-		
60	57	IP	M	surgery	wound infection	?2 weeks	diabetes	R	R	R	R(D test)	R	R	R	R	S	I	S	S	+	+	20.08
61	25	IP	F	OG	surgical site inf	?2 weeks	surgery	S	S	S	S	S	S	R	R	S	I	S	S	-		
62	30	IP	M	ortho	surgical site inf	?2 weeks	surgery	R	S	I	S	S	S	R	R	S	S	S	S	-		
63	55	OP	F	ortho	surgical site inf	-	surgery	R	R	R	S	R	R	R	R	S	I	S	S	+	+	36.56
64	67	OP	M	surgery	carbuncle	-	diabetes	R	R	R	S	R	I	R	I	S	I	S	S	+	+	20.03
65	16	IP	F	ENT	ear discharge	?2 weeks	-	R	S	I	I	I	S	R	I	S	I	S	S	-		
66	11	OP	M	ENT	ear discharge	-	-	R	S	I	I	R	S	R	I	S	I	S	S	-		
67	29	IP	M	ortho	surgical site inf	?2 weeks	surgery	R	S	S	S	S	S	S	S	S	I	S	S	-		
68	45	IP	F	ENT	ear discharge	?2 weeks	-	R	S	I	S	S	S	R	I	S	I	S	S	-		
69	12	IP	M	ENT	ear discharge	?2 weeks	-	R	R	I	S	R	S	R	R	S	S	S	S	+	+	17.14
70	19	IP	M	surgery	wound infection	?2 weeks	-	R	S	I	S	S	S	S	S	S	I	S	S	-		
71	1	IP	F	paediatrics	abscess	?2 weeks	-	R	S	R	S	S	S	S	S	S	I	S	S	-		
72	65	IP	F	medicine	abscess	?2 weeks	diabetes	R	S	S	I	S	R	I	R	S	S	S	S	-		

73	15	IP	F	medicine	abscess	⍰2 weeks	-	R	S	I	S	S	S	I	I	S	I	S	S	-		
74	60	IP	M	ortho	surgical site inf	⍰2 weeks	surgery	R	S	I	I	S	R	I	R	S	S	S	S	-		
75	25	IP	M	ENT	ear discharge	⍰2 weeks	-	R	R	R	I	R	S	R	R	S	S	S	S	+	+	15.03
76	1	IP	M	paediatrics	wound infection	⍰2 weeks	-	R	R	R	R	R	R	R	R	S	I	S	S	+	+	15.4
77	35	IP	F	OG	surgical site inf	⍰2 weeks	surgery	R	R	R	R	R	R	I	I	S	I	S	S	+	+	36.05
78	82	IP	M	skin	boil	⍰2 weeks	-	R	S	I	S	S	S	R	R	S	S	S	S	-		
79	14	IP	F	surgery	surgical site inf	⍰2 weeks	surgery	R	S	I	S	S	S	R	R	S	I	S	S	-		
80	31	IP	F	ENT	ear discharge	⍰2 weeks	-	R	R	I	I	R	I	I	I	S	I	S	S	+	+	16.65
81	47	IP	M	ortho	surgical site inf	⍰2 weeks	surgery	R	S	I	S	S	S	R	S	S	S	S	S	-		
82	48	IP	F	surgery	surgical site inf	⍰2 weeks	diabetes	R	S	R	S	S	I	R	R	S	S	S	S	-		
83	12	IP	M	paediatrics	wound infection	⍰2 weeks	-	R	R	R	R	R	R	I	S	S	S	S	S	+	+	18.61
84	24	IP	F	OG	surgical site inf	⍰2 weeks	surgery	R	R	R	I	I	S	R	S	S	I	S	S	+	+	15.38
85	35	IP	M	surgery	wound infection	⍰2 weeks	-	R	S	I	I	S	S	R	R	S	I	S	S	-		
86	40	IP	M	surgery	surgical site inf	⍰2 weeks	surgery	R	S	I	S	S	S	I	I	S	I	S	S	-		
87	60	IP	M	ortho	surgical site inf	⍰2 weeks	surgery	R	S	I	S	S	S	I	S	S	I	S	S	-		
88	70	IP	M	surgery	wound infection	⍰2 weeks	-	R	S	S	S	S	S	I	S	S	S	S	S	-		
89	52	IP	M	skin	wound infection	⍰2 weeks	-	R	R	I	S	R	S	I	I	S	S	S	S	+	+	14.47
90	36	IP	M	skin	boil	⍰2 weeks	-	R	R	R	R(D test)	R	I	R	I	S	S	S	S	+	+	16.7
91	3	IP	F	paediatrics	burns	⍰2 weeks	burns	R	S	S	I	S	S	S	S	S	S	S	S	-		
92	47	IP	F	ortho	surgical site inf	⍰2 weeks	surgery	R	S	I	I	S	S	S	S	S	S	S	S	-		
93	45	IP	M	skin	boil	⍰2 weeks	HIV	R	R	I	S	R	S	I	S	S	S	S	S	+	+	15.79
94	5	IP	M	paediatrics	abscess	⍰2 weeks	-	R	S	I	I	S	S	I	S	S	S	S	S	-		
95	26	IP	F	OG	surgical site inf	⍰2 weeks	surgery	R	S	S	S	S	S	I	R	S	S	S	S	-		
96	1	IP	M	paediatrics	wound infection	⍰2 weeks	-	R	R	R	R	R	R	R	R	S	S	S	S	+	+	21.15
97	67	IP	M	ENT	ear discharge	⍰2 weeks	-	R	S	I	S	R	I	R	I	S	S	S	S	-		
98	43	IP	M	surgery	wound infection	⍰2 weeks	diabetes	S	S	S	S	S	S	S	S	S	S	S	S	-		
99	3	IP	F	paediatrics	boil	⍰2 weeks	-	S	S	S	S	S	S	I	S	S	S	S	S	-		
100	68	IP	M	ortho	surgical site inf	⍰2 weeks	surgery	R	S	I	S	I	S	I	R	S	S	S	S	-		

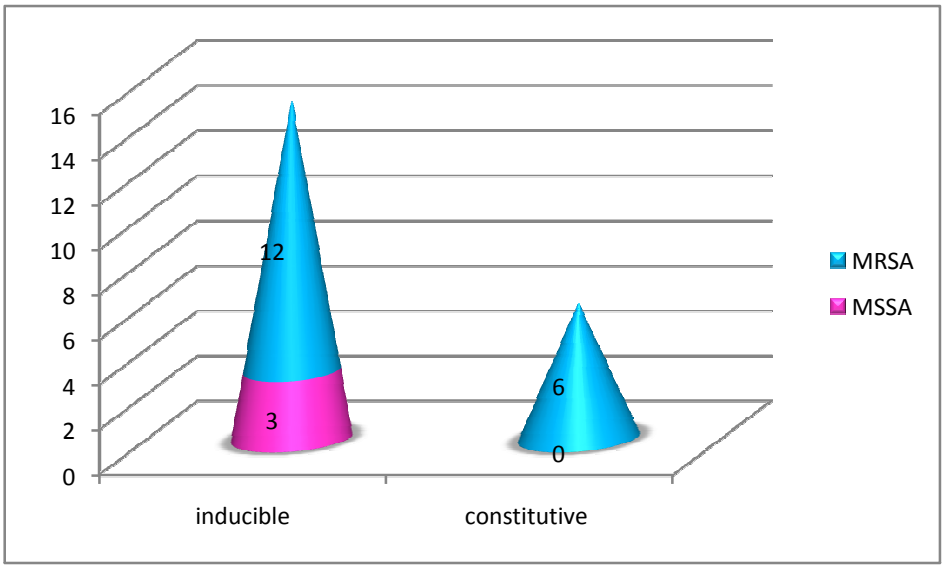
	MRSA
<2 weeks	78.1
>2 weeks	21.9

To resize chart data range, drag lower right corner of range.

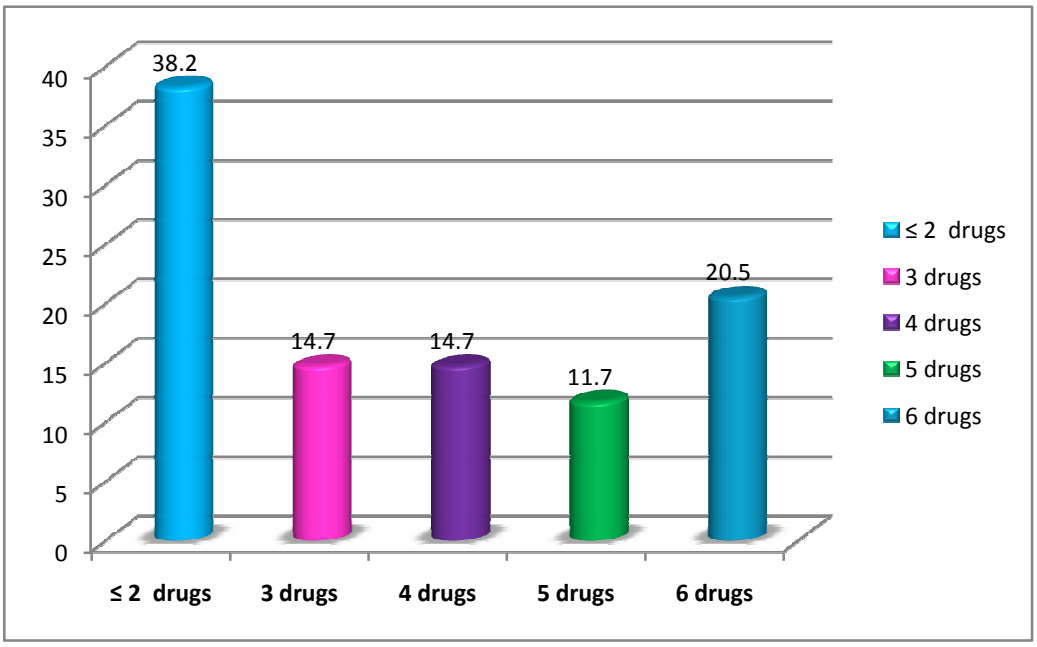
Risk factors	Pecentage
Surgery	26.5
Diabetes	11.8
Burns	2.9
Job's syndrome	2.9
HIV	2.9
Unidentified	52.9
Total	100



	inducible	constitutive
MSSA	3	0
MRSA	12	6



	MRSA
≤ 2 drugs	38.2
3 drugs	14.7
4 drugs	14.7
5 drugs	11.7
6 drugs	20.5



ABBREVIATIONS

S.aureus	-	*Staphylococcus aureus*
MRSA	-	Methicillin Resistant *Staphylococcus aureus*
MSSA	-	Methicillin Sensitive *Staphylococcus aureus*
HA-MRSA	-	Hospital acquired MRSA
CA-MRSA	-	Community acquired MRSA
PBP	-	Penicillin Binding Protein
SCC*mec*	-	Staphyloccocal Cassette Chromosome *mec*
PVL	-	Panton-Valentine Leucocidin
CLSI	-	Clinical Laboratory Standards Institute
DD test	-	Disc diffusion test
PCR	-	Polymerase Chain Reaction
NPV	-	Negative Predictive Value
PPV	-	Positive Predictive Value
Ct value	-	Cross threshold value

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Annexure – 1

(Media Preparation)

Annexure –I

(Media preparation)

1.Preparation of Mueller-Hinton agar

Composition		gms/l
Beef infusion, from	-	300
Casein acid hydrolysate	-	17.5
Starch	-	1.5
Agar	-	17.00
Final p^H at 25˚C	-	7.3±0.1

Preparation

Suspend 38.0 grams in 100 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121˚C) for 15 minutes. Mix well before pouring.

2. Preparation of HiCrome MeReSa agar

For the isolation and selective identification of Methicillin Resistant *Staphylococcus aureus* (MRSA) from clinical isolates.

Ingredients		gms/l
Casein enzymic hydrolysate	-	13.00
Yeast extract	-	2.50
Beef extract	-	2.50
Sodium pyruvate	-	5.00
Sodium chloride	-	40.00

Chromogenic mixture	-	5.30
Agar	-	15.00

Final p^H (at 25°C) 7.0 ± 0.2

Directions

Suspend 41.65 grams in 500 ml distilled water. Heat to boiling to dissolve the medium completely. Do not autoclave. Cool to 45-50°C. Aseptically add sterile rehydrated contents of 1 vial of MeReSa Selective Supplement. Mix well and pour into sterile petri plates.

Annexure –2

(Proforma of the Data sheet)

ANNEXURE II

DATA SHEET FOR COLLECTION OF SOCIO DEMOGRAPHIC, CLINICAL AND LABORATORY DATA FOR P.G. DISSERTATION WORK ON "EVALUATION OF CHROMAGAR AND PCR FOR DETECTION OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) FROM CLINICAL ISOLATES"

Serial no:

Name : Lab no:

Age/sex: IP no:

Address: Ward:

HISTORY

1.Chief complaint :

2. H/o Surgery/ RTA/Burns/Others:

3. Duration of hospital stay :

4. H/o Diabetes/Steroids intake/Chemotherapy/Others:

GENERAL EXAMINATION

Built : Pulse:

Nourishment: BP:

Pallor:

Temperature:

LOCAL EXAMINATION

Site :

Induration:

Size:

Erythema:

PURULENT DISCHARGE

Amount:

Type: purulent/ blood stained/others

Colour :

Odour:

INVESTIGATIONS

Specimen received - Pus

1.CULTURE

Nutrient agar	Mac Conkey agar	Blood agar

2.MICROSCOPY

3.BIOCHEMICAL REACTIONS

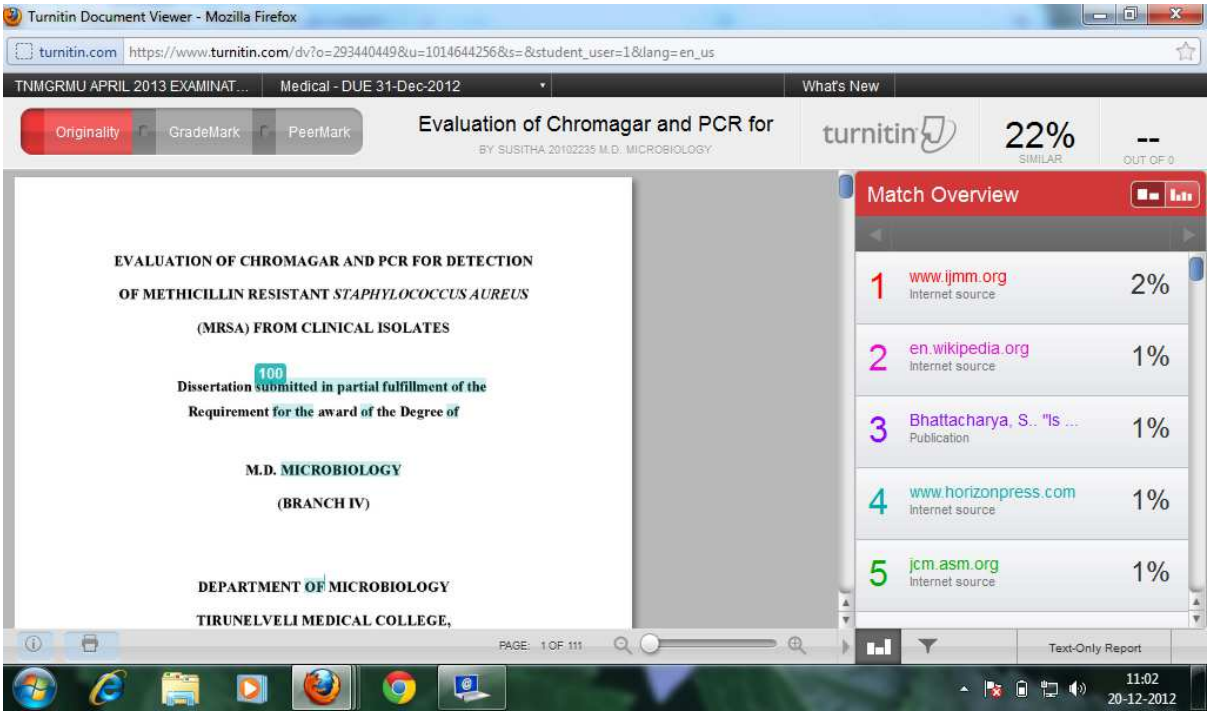
Catalase test	Slide Coagulase test	Tube Coagulase test

4.ANTIBIOGRAM

Drug	Zone size	Interpretation
Penicillin		
Cefoxitin		
Erythromycin		
Clindamycin		
Ciprofloxacin		
Gentamicin		
Amikacin		
Cotrimoxazole		
Vancomycin		
Teicoplanin		
Linezolid		
Tigecyline		
Furazolidone		

5.CHROM agar:

6.mec A gene :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